PB82-143447

Authorhythmometry

Minnesota Univ., Minneapolis

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Dec 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing) XX-81-077

1. REPORT NO. EPA-600/J-76-110	2. JOURNAL ARTICLE	3. RECIPIENT'S ACCESSION NO. PB82-143447
4. TITLE AND SUBTITLE Authorhythmometry		5. REPORT DATE December 1981
		6. PERFORMING ORGANIZATION CODE W362025
7. AUTHOR(S) Franz Halberg, Renato Lauro, and Franca Carandente		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Chronobiology Laboratories University of Minnesota Minneapolis, Minnesota 55455		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. Grant R-804512
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711 (RTP,NC)		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES
Published In: La Riceria in Clinica e in Laboratorio 6(3): 207-250, 1976 (COLOR ILLUSTRATIONS REPRODUCED IN BLACK & WHITE)

16. ABSTRACT

The authors stress the importance of rhythmometry, thanks to which the time course and other peculiarities of any biological phenomenon can be evaluated by obtaining serial measurements, objectively quantifying their characteristic features, and working out special models with the aid of computers. Of special interest, particularly for the evaluation of reference standards, is autorhythmometry (AR), to be used at least for certain variables. This is the method by which each subject studies himself, performing a certain number of measurements in the course of the day (or month or year) of his body temperature, blood pressure, cardiac rhythm, grip strength, etc. In AR, the subject takes an active part in the study of his condition of healthand it has been shown that in the majority of cases these measurements are done with the utmost accuracy and precision, providing that the purpose of the operations to be performed has been adequately explained. The wide spread use of AR, during more of less extended periods in a lifetime, would permit the assessment of the normal situation for eash individual and thus offer the possibility of diagnosing any disorder at its very beginning; it would thus be of great prophylactic value. The authors refer to the example of arterial blood pressure; widely different values for the normal levels can be found in the texts of different authors. They also show that a certain pressure may be normal for a given subject at a certain time of day and be a sign of illness for another subject, or even for the same subject at a different time. The authors suggest the introduction of AR in the curriculum of secondary schools and into the routine for outpatients and inpatients. This practice would increase the availability of information on public health at a lower cost.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 47
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

PB82-143447

EPA-600/J-76-110
Journal Article

reprint from
LA RICERCA in Clinica e in Laboratorio Vol. VI, July-September 1976 - number 3

Chronobiology Laboratories - Department of Laboratory Medicine and Pathology
University of Minnesota, Minneapolis, Minnesota 55455, USA

Clinica Medica Generale e Terapia Medica II
Università di Roma, Italia

Clinica Medica Generale e Terapia Medica III
Università di Milano, Italia

AUTORHYTHMOMETRY

Leads from Single-Sample Medical Check-Ups toward a Health Science of Time Series *

FRANZ HALBERG RENATO LAURO FRANCA CARANDENTE

* *Supported by the US Public Health Service (5-K6-GM-13,981), National Cancer Institute (1R01-CA-14445-01), Cancer Supplement (CA-14445-01S1), US Environmental Protection Agency and Hoechst Foundation.*

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

Chronobiology Laboratories - Department of Laboratory Medicine and Pathology
University of Minnesota, Minneapolis, Minnesota 55455, USA

Clinica Medica Generale e Terapia Medica II
Università di Roma, Italia

Clinica Medica Generale e Terapia Medica III
Università di Milano, Italia

AUTORHYTHMOMETRY

Leads from Single-Sample Medical Check-Ups toward a Health Science of Time Series *

FRANZ HALBERG RENATO LAURO FRANCA CARANDENTE

Biorhythms and health

Rhythms [2, 9, 27, 34, 36, 63, 64, 73, 76] relate to the health of an individual and of an entire population, topics of interest and study on the part of scientists, politicians, philosophers and sociologists [70]. The World Health Organization regards health as 'a condition of complete physical, mental and social well being and not solely as the absence of disease and infirmity' [92]. Such a statement, in itself, does not suffice to describe health quantitatively as one would expect a scientific approach to do and indeed we are dealing with a statement of principles rather than a definition [86].

At this time even a semi-quantitative assessment of health is lacking. In confronting descriptions of '. . . the range and levels of health and illness, extending from zero health (death) at one end of a *continuum* to optimal health at the other end', one finds that any quantitative distinction between various levels of health and illness is difficult to make, except in the case of death [53].

The scope of the conventional approach has been aptly described by LOGAN [62]: '. . . What is usually done in estimating and comparing the health of populations is to measure the deaths from various causes, the extent to which death is avoided, the absence of a demand for medical care, or at least the absence of an obvious need for medical care'.

All of these criteria are negative ones; moreover, all of them are population-based. Accordingly, they require methodology consisting of the statistical assessment, in big population samples, of mortality, morbidity or some other phenomenon related to these conditions. In this fashion, one can estimate only indirectly the individual's chances to fall sick or to die. A direct evaluation of the health of the single person is not available, nor does one obtain individualized information which serves as a basis for preventive medicine or therapeutic action.

Key-words: Autorhythmometry; Blood pressure; Circadian; Health; Rhythm; Time series.

** Supported by the US Public Health Service (5-K6-GM-13,981), National Cancer Institute (1R01-CA-14445-01), Cancer Supplement (CA-14445-01S1), US Environmental Protection Agency and Hoechst Foundation.*

Received, October 25, 1974 and modified in February, 1976.
La Ricerca Clin. Lab. 6, 207, 1976.

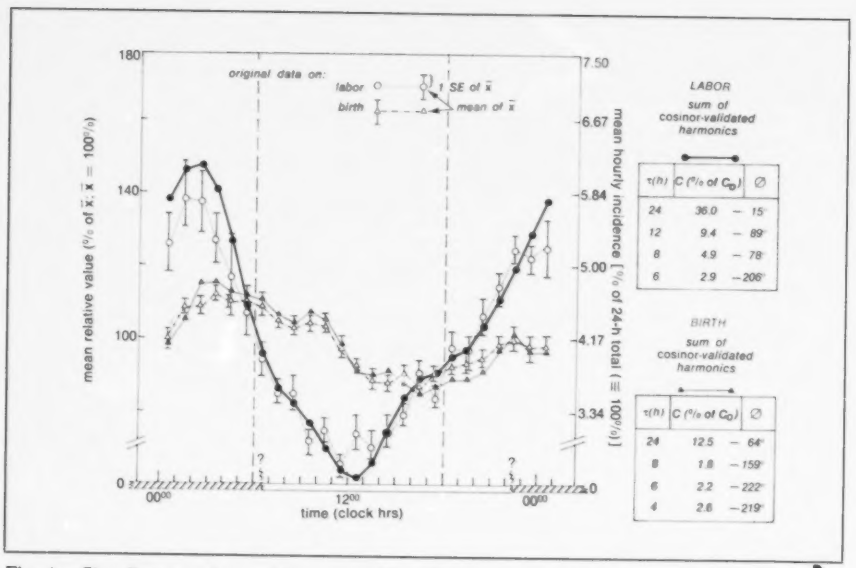

original data on:
labor
birth
1 SE of x̄
mean of x̄
mean relative value (% of x̄; x̄ = 100%)
mean hourly incidence [% of 24-h total (≡ 100%)]
time (clock hrs)
LABOR
sum of cosinor-validated harmonics
τ(h) | C (% of C0) | ∅
24 | 36.0 | − 15°
12 | 9.4 | − 89°
8 | 4.9 | − 78°
6 | 2.9 | −206°
BIRTH
sum of cosinor-validated harmonics
τ(h) | C (% of C0) | ∅
24 | 12.5 | − 64°
8 | 1.8 | −159°
6 | 2.2 | −222°
4 | 2.8 | −219°

Fig. 1 - Circadian waveform of human natality. Chronograms of human natality — spontaneous initiation of labor and natural birth — characterized by high amplitude circadian as well as lower amplitude ultradian frequencies. Comparison of the circadian waveform, constructed from significant periodicities validated by *cosinor*, with original data is good. Note blue regions at bottom of figure approximate assumed nocturnal rest spans.

All in all, it seems desirable, as soon as possible, to replace current views of disease based on an essentially negative line of thought and action; we need a definition of health which is positive, individualized, realistic (since it is economical to implement), yet amenable to quantification [27]. Thereby, one would greatly extend the scope of MORIYAMA's [66] *desiderata* that the data for the definition be obtainable if not available while keeping in mind the remainder of Moriyama's goals, including meaningfulness, comprehensibility, sensitivity, collection of data on the basis of clear, justifiable and reasonable assumptions, and description of clearly defined components that contribute independently to the total complex data.

Equally important, if not more so, is yet the elimination of the current misconception that basically the body is regulated for constancy and that a fictitious 'equilibrium' is a *sine qua non* for the maintenance of health.

This concept of homeostasis, appealing as a broad generalization, admits by implication (or in small print) the occurrence of variability; yet, in practice, the very emphasis on regulation for constancy does not usually include concern for the predictable components of human variability.

More often than not, homeostasis, in sanctioning the complete disregard of (predictable) rhythms in practice, may well be at the root of what is currently a medical *art* based on *single samples*. The evidence is overwhelming for the proposition, recognized already by Claude BERNARD [23], that the organism is characterized by an extreme variability [8]. However, Bernard was unable to deal with biologic phenomena requiring inferential statistical approaches; he did not estimate for a given result the role(s) played by uncontrolled (known or unknown) or uncontrollable variability. Bernard therefore ignored the fact that man and many other life forms are characterized by a variability that contains *resolvable* spontaneous rhythms of a predictably

dramatic extent, in many entities, from society (figs 1 and 2) [29, 75, 88] to cell (fig. 3) [34, 38, 48].

The recognition of a spectrum of frequencies characterizing so many functions (fig. 4) [3, 41, 52, 65] underlies a dynamic concept of health. This view includes focus upon the individual's predictably changing ability to react properly to both usual and unusual stimuli by adjustments in the extent or timing of rhythms. Indeed, as the chronobiologist resolves predictable, spontaneous changes, he also estimates the nature and the extent of responses to environmental factors. Thus eventually, as a dynamic approach to the problem of health assessment and maintenance, he may be able to optimize the timing of activities, in work and leisure: for best immediate productivity compatible with long-term health.

It is the chronobiologist's task, further, to make possible the systematic exploitation of an organism's time structure by developing inexpensive yet objective methods of measurement that do not depend upon costly technology. At the outset, this task seems formidable. However, even though more conventional single potshot screening — e.g. for blood pressure elevation — have not yet exploited time series analysis and have not been themselves generally accepted, cost-benefit and other social considerations do not rule out rhythm evaluation for health assessment and preventive medicine if thereby one can get 'more for less', as will be indicated below.

Currently, a wealth of serial observations continuously monitored in intensive care wards is almost invariably discarded. On a few occasions, however, time series from coronary care wards have been saved for further analyses and suffice to demon-

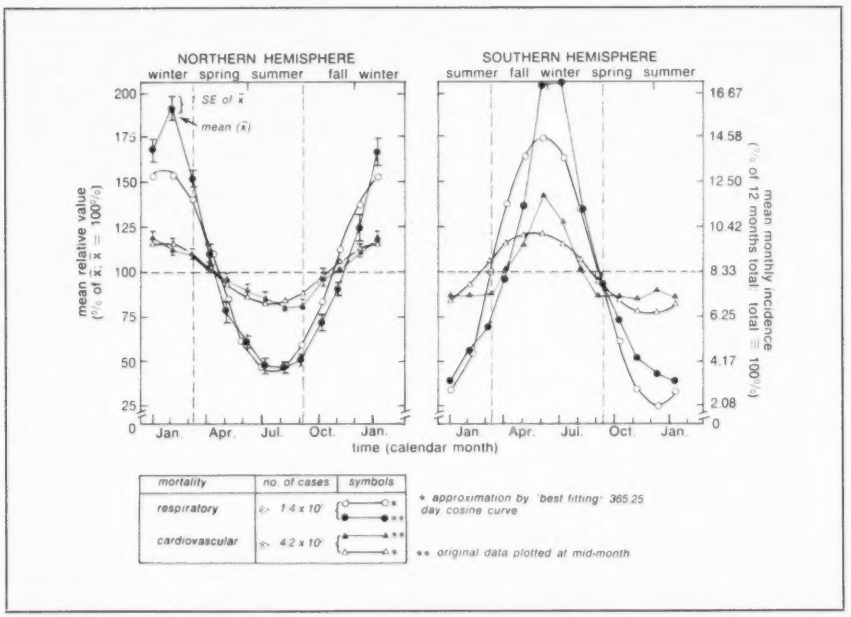


Fig. 2 - Circannual chronogram of human respiratory and cardiovascular deaths. Chronograms of human mortality population rhythms reveal highest incidences during winter irrespective of hemisphere. Least squares approximation of the monthly means by a 365.25-day cosine curve accurately reflects waveforms for the Northern hemisphere. Slight discrepancies between original data and approximating curve for the Southern hemisphere may be due to small number of samples available.

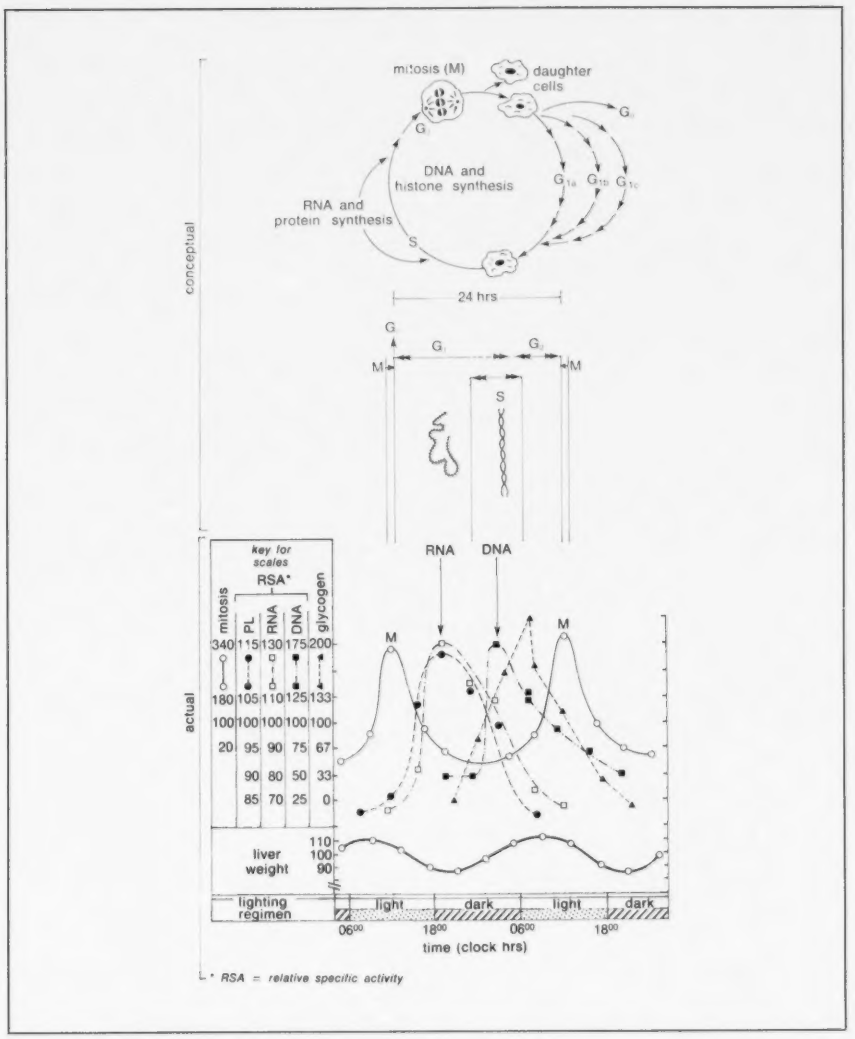


Fig. 3 - Circadian stages of 24-h synchronized events in the cell cycle. Different cellular processes in growing mouse liver: labeling of phospholipids (PL), RNA and DNA, glycogen content and mitosis (as well as liver weight) in relation to a regimen of alternating light and darkness. Sensitive stages of the cell cycle may become directly amenable to therapeutic exploitation by timing carcinostatic treatment according to rhythms. A simplified 'classical' cell cycle, as sketched *inter alios* by MUELLER G. C. (Fed. Proc. *28*, 1780, 1969), may well be aligned for this purpose with the actual cellular circadian system here demonstrated for immature growing mouse liver. Similar circadian and other rhythmicity (with definable though not the same timing) characterizes many tissues of the mammalian host with neoplasia and should be exploited for timed treatment. (From: HALBERG F. et al. [34]; HALBERG F.: Perspect. Biol. Med. *3*, 491, 1960).

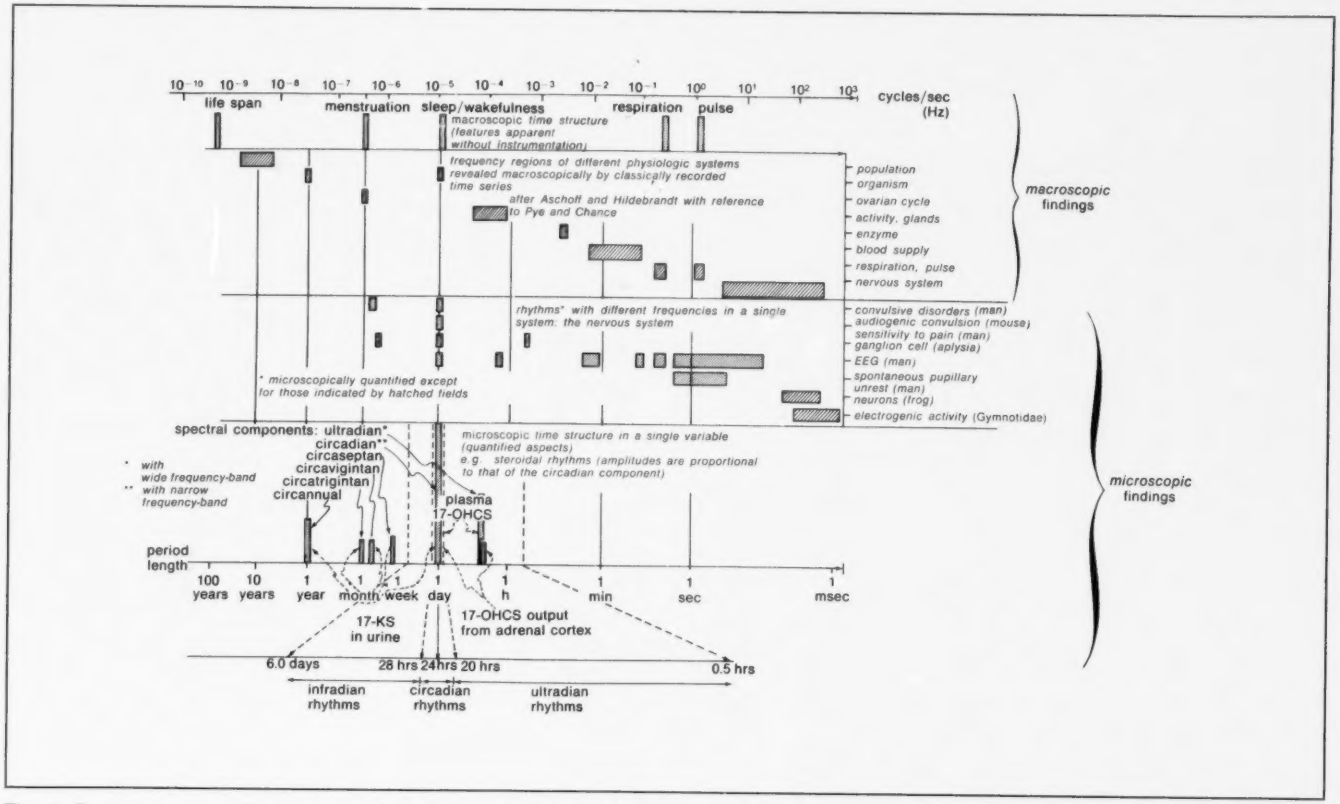


Fig. 4 - From overt temporal parameters and *macroscopically* different 'frequency-regions' of different physiologic systems to several *microscopically* quantified rhythms in a single system or even in a single variable.

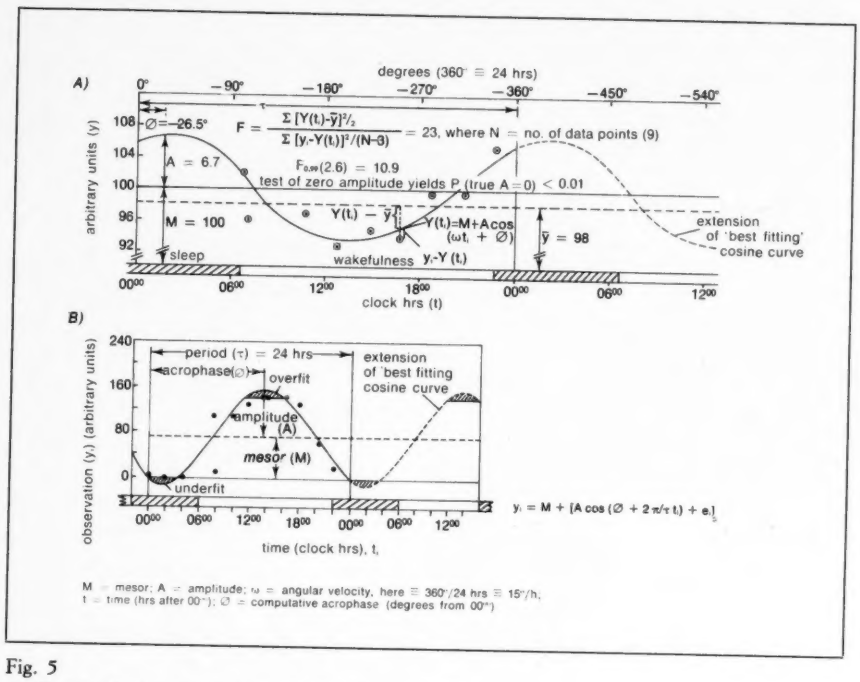


Fig. 5

A) Testing rhythm sinusoidality by variance ratio, F;
abstract example with 24-h cosine function y(t), continuous curve, fitted by least squares to data $y_i(o)$ obtained during wakefulness span;
M = mesor; A = amplitude; ω = angular velocity, here ≡ 360°/24 hrs ≡ 15°/h; t = time (hrs after 00⁰⁰); Ø = computative acrophase (degrees from 00⁰⁰); ω = 2π/τ.

B) Parameter M, A and Ø estimation by least squares fit of cosine model with fixed period:
$y_i = M + [A \cos (\varnothing + 2\pi/\tau t_i) + e_i]$;
t_i = time; y_i = observation at t_i; e_i = error at t_i, having an independent normal distribution with mean zero and unknown variance σ^2.

Computer programs facilitate inferential statistical rhythm detection in noisy data such as those usually collected in a clinic (*A*) and the estimation of the rhythm's properties (*B*).

strate rhythms with several frequencies not only in heart rate but even in the frequency of cardiac rhythm alteration, such as premature ventricular beats [59]. Electrocardiograms or electroencephalograms, when continuously recorded along the 24-h scale from human or nonhuman primates and analyzed for consecutive 5- or 6-min spans, reveal for the more specific rhythms of the heart or brain action potentials both a circadian (about 24-h) and a yet higher-frequency (ultradian) modulation [15, 41, 59].

With rather sparse data, chronobiologists have not only succeeded in demonstrating rhythms for an ecological system, a population, a group, an individual or one of the many subdivisions of such entities, e.g. organs, tissues, cells or subcellular elements, but have also quantified such rhythms in terms of their prominent features, (fig. 5) [24, 77]. Each day, in health, our adrenal cortical hormones reach values comparable to those found in the condition of adrenal insufficiency, while 12 hrs before or later the blood concentration of the same hormone is comparable to that found in Cushing's disease [36, 64]. These impressive and somewhat predictable changes of adre-

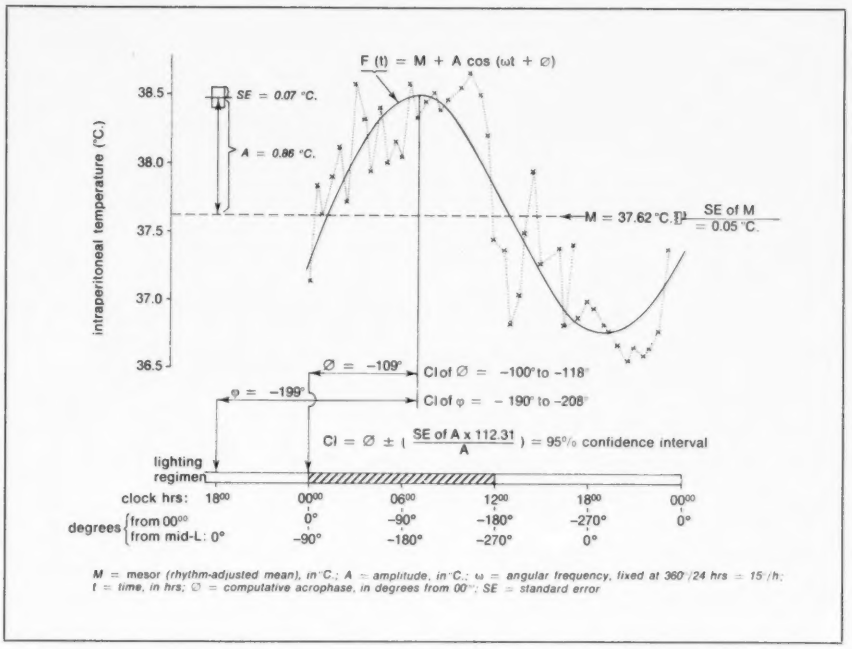

F (t) = M + A cos (ωt + ∅)
SE = 0.07 °C.
A = 0.86 °C.
M = 37.62 °C.
SE of M = 0.05 °C.
intraperitoneal temperature (°C.)
38.5
38.0
37.5
37.0
36.5
∅ = −109°
CI of ∅ = −100° to −118°
φ = −199°
CI of φ = −190° to −208°
CI = ∅ ± (SE of A x 112.31 / A) = 95% confidence interval
lighting regimen
clock hrs: 18⁰⁰ 00⁰⁰ 06⁰⁰ 12⁰⁰ 18⁰⁰ 00⁰⁰
degrees from 00⁰⁰: 0° −90° −180° −270° 0°
degrees from mid-L: 0° −90° −180° −270° 0°
M = mesor (rhythm-adjusted mean), in °C.; A = amplitude, in °C.; ω = angular frequency, fixed at 360°/24 hrs = 15°/h;
t = time, in hrs; ∅ = computative acrophase, in degrees from 00⁰⁰; SE = standard error

Fig. 6 - Least squares fit of 24-h cosine function (continuous line) to intraperitoneal temperatures (x) of an adult female MSD rat. (A first step to identify rhythms as the periodic characteristics of the data under examination, is to detect *mesor*, *amplitude* and *acrophase* by the least squares method; the parameters obtained are not the final results but only intermediate values. We can see here the two different acrophases: computative and external — the internal acrophase is not indicated). Telemetry at ~30 min intervals for 24 hrs.

domain; region	*range*
ultradian	$\tau < 20$-h
circadian	20-h $\leq \tau \leq$ 28-h
dian	23.9-h $\leq \tau \leq$ 24.1-h
infradian	$\tau >$ 28-h
circaseptan	$\tau = 7 \pm 3$ d
circadiseptan	$\tau = 14 \pm 3$ d
circavigintan	$\tau = 21 \pm 3$ d
circatrigintan	$\tau = 30 \pm 5$ d
circannual	$\tau = 1$ y ± 2 m

τ = period; h = hour; d = day; m = month; y = year.

Table 1 - Period ranges for terms describing biologic rhythms.

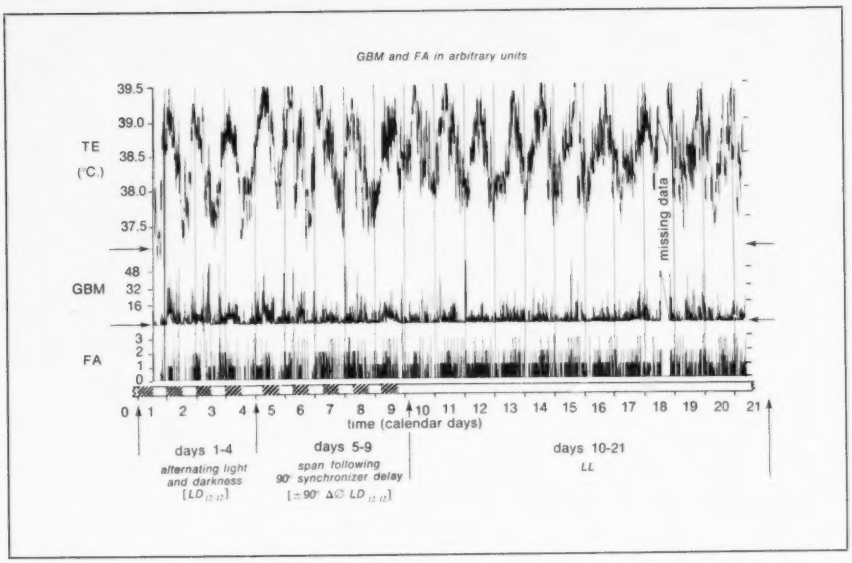


Fig. 7 - Clipped chronogram of telemetered intraperitoneal temperature (TE), gross body movement (GBM), and feeding activity (FA), of mature female CFE rat. GBM and FA in arbitrary units.

nal function are associated in man and mouse with modifications in the counts of certain leukocytes. As does the organism as a whole, even the metabolism of DNA undergoes a circadian rhythm [6, 34].

A recent study [10] on *spontaneously mesor hypertensive Okamoto rats* showed that the susceptibility of the gastric mucosa to ulcerogenesis undergoes a circadian rhythm; thus, in one and the same inbred rat strain, a model has become available for the chronobiologic study of multiple risks . . . gastric ulcerogenesis, the consequences of blood pressure elevation and even cancer. Work on this model seems to be most promising from the social point of view; for shift workers, a particular predisposition to gastrointestinal disease and blood pressure elevation has been noted.

Thus, human life follows a set of schedules determined by both external and internal influences. The result of interactions between an organism and its ambience are the biological rhythms, the temporal changes in physiologic parameters which exhibit a reproducible waveform [24]. Once rhythms are identified as the periodic characteristics of our data, they may be measured according to several parameters, figs 5 and 6: period, amplitude (a measure of the extent of rhythmic change), acrophase (a measure of timing; the lag from a reference time of the crest in the function used to approximate all data), waveform, and *mesor* (a rhythm-determined average).

Some aspects of rhythms with a frequency of about 1 cycle/day (circadian rhythms) are innate and so are some features of rhythms with about 1 cycle/month, 1 cycle/year and even 1 cycle/week [11, 33, 45], tab. 1. As spectral bands, rather than lines, these rhythms characterize the species rather than solely the individual. Many circadian rhythms persist under conditions of constancy instituted in the environment for experimental animals with respect to, say, temperature and lighting, fig. 7 [2, 43], and they exhibit an undamped amplitude within a species-characteristic band, at a frequency that varies from animal to animal and has no known environmental counterpart [2, 24, 28, 43]. Even in the face of continued maintenance in a periodic environment,

organism	variable	extent of shift in synchronizing lighting regimen		hrs to adjust to regimen			references
		hrs (on a 24-h cycle)	degrees (360° = 24 hrs; 15° = 1 h)	advance	delay	difference	
				faster advance			
Albizzia julibrissin (silk tree)	pinnule angle	4 *	60	~ 24	~ 52	28	KOUKKARI and HALBERG [58]
Fringilla coelebs (chaffinch)	jumping activity	6	90	~ 60	~120	60	ASCHOFF and WEVER [5]
				faster delay			
Tribolium confusum (flour beetle)	O_2 consumption	6	90	> 48	> 24	24	CHIBA et al. [12]
Sprague-Dawley rat	intraperitoneal temperature	6	90	~312	~120	192	HALBERG et al. [43]
monkey (several species)	axillary temperature	6	90	>120	~ 72	48	HALBERG [26, 28]
man	oral temperature **	6	90	~312	~192	120	HALBERG [24]

* 4 hrs = 1/2 the dark span in *Albizzia*. 6 hrs = 1/2 the dark span in rat, monkey, and chaffinch;
** in social setting. Studies by ASCHOFF [4] on men isolated in a bunker reveal faster advance than delay.

Table 2 - Comparative physiologic studies of 24-h-synchronized circadian rhythms reveal structured adjustment in the form of differences in rates of rhythm advance and delay.

new frequencies can come to the fore, fig. 8. Frequencies consistently (yet only slightly) higher than 1 cycle/day are seen following but one manipulation, such as the removal of the eye in the case of the rectal temperature rhythm of certain mice [28].

If rhythms constituted conditioned reflexes impressed, for instance, by the lighting schedule, they should disappear eventually; they do not.

Under conditions of synchronization with a cycle of darkness and light, this schedule can serve to manipulate the timing of rhythms in animals with eyes but not in those without eyes [28]. A change in timing of rhythms in a number of variables of mice and men [1, 30, 69] also may be achieved, against a fixed lighting regimen, by manipulating meal timing.

Even the internal timing of circadian rhythms thus can be altered, since rhythms in different variables of the same organism can be differently synchronized, modulated or at least influenced by the interaction of several factors, socio-ecological and/or geophysical. Rhythms that are amenable to modifications by meal timing may not disappear in the absence of this synchronizer cycle [13, 25]. Another characteristic of these rhythms is the directedness (polarity or asymmetry) of their adjustment, summarized in tab. 2 [60] for several life forms. Figs 9, 10 and 11 document shifts of rhythm timing for mice, rats and human beings. Thus, rhythms are not only ubiquitous and persistent features of our physiology, they are also amenable to modification of several of their characteristics. One of these modifiable characteristics is the rhythms' timing. Thus arises the opportunity for optimizing activities (medical, ecological, educative or other), according to the most appropriate stages of temporal structure.

It can be documented under controlled laboratory conditions that rhythms are critically important aspects of the life processes of organisms. The mammalian response to a stimulus is neither constant nor randomly variable; it is predictably rhythmic along several time scales, of 24 hrs, about 1 week, about 1 month or 1 year.

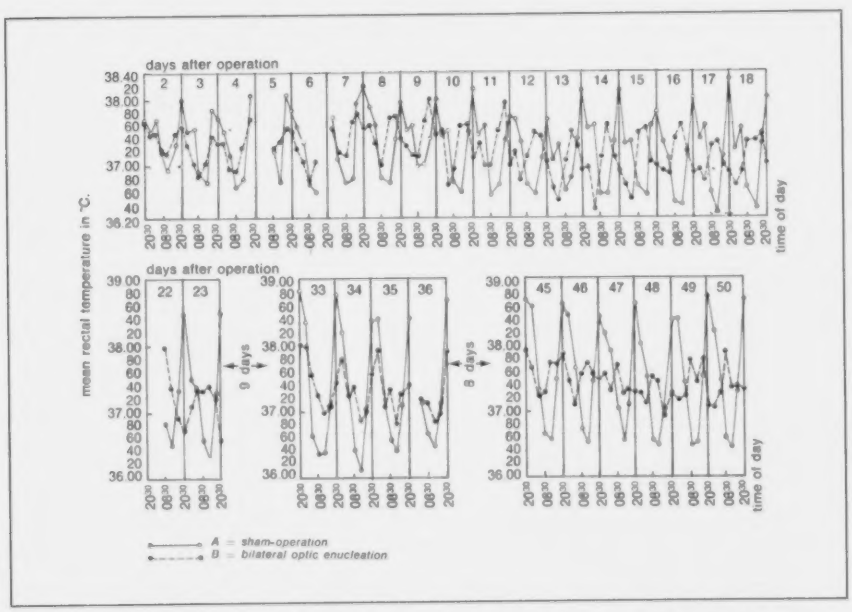


Fig. 8a - *Macroscopic* circadian desynchronization in mice after bilateral optic enucleation.

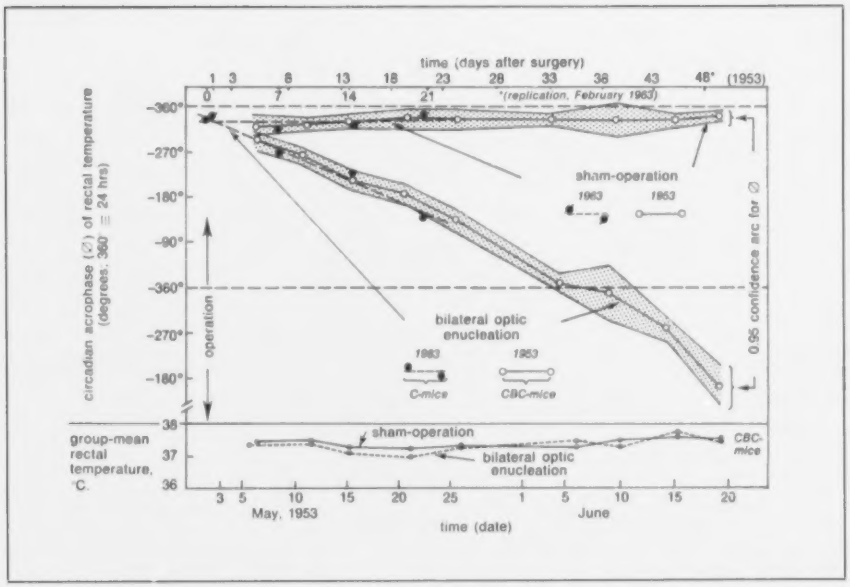


Fig. 8b - Acrophase-drift of desynchronized circadian rhythm in body temperature of blinded mature CBC and C mice compared with 24-h-synchronized circadian rhythm of sham-operated 'controls'.

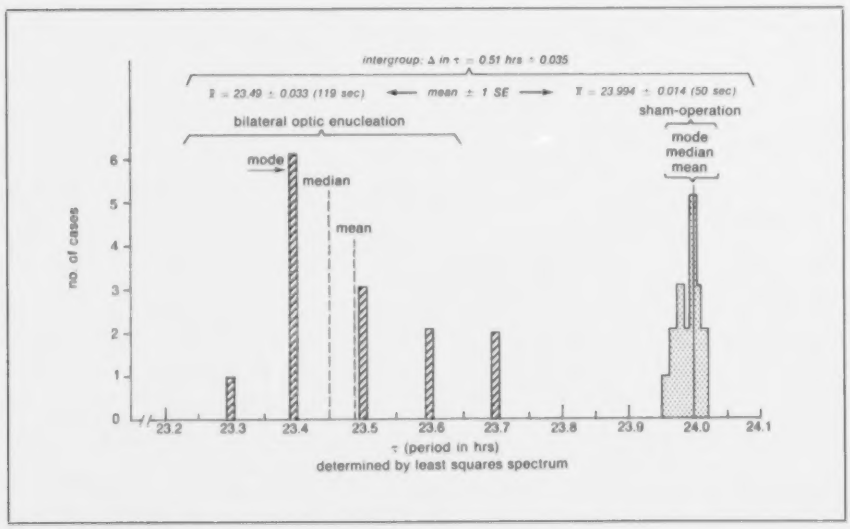


Fig. 8c - Circadian period of rectal temperature in 14 blinded and 18 sham-operated CBC mice (1953-series).

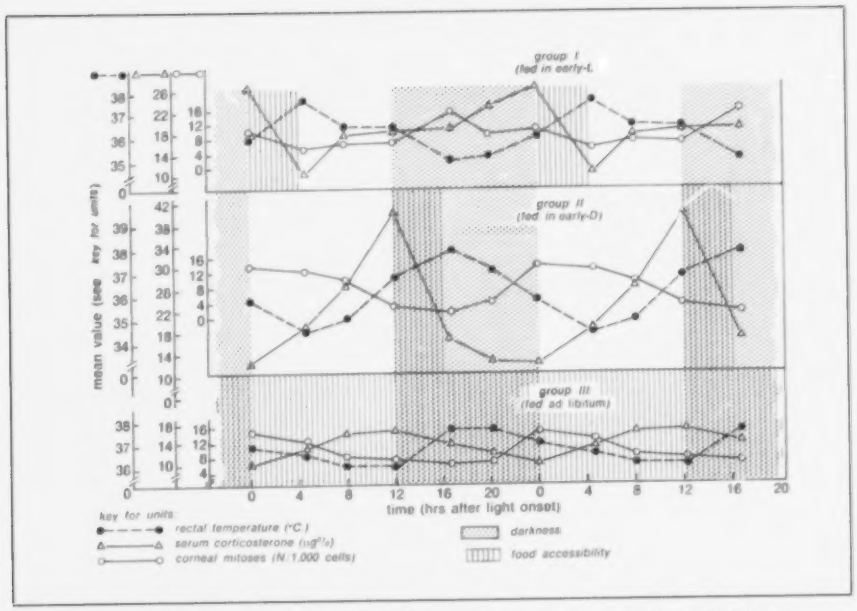


Fig. 9 - Relations of circadian rhythmic variables in mice on different feeding schedules.

Predictable circadian rhythms account for different reactions of an organism to stimuli such as X-rays [50], drugs [6, 77] and bacterial endotoxins [39, 89]. Findings on these so-called hours of changing resistance have been extended to man by rigorous studies along the 24-h scale involving the administration of a controlled stimulus at different times.

The importance of a statistically manifested about-yearly change in vulnerability stands out in statements by Hippocrates: 'While all diseases are likely to occur at all times, certain diseases are more likely to occur in certain seasons' [56]. Mortality statistics along the 1-year scale, fig. 2, and along the 24-h scale, fig. 12, show that Hippocrates was right. For deaths from certain conditions, one can estimate even differences in the timing of their peaks. Thus, during 27 years (fig. 13), suicides in Minnesota showed a peak about May, while during the same span, deaths from heart disease peaked much earlier in the calendar year [29]. Quite clearly, the factors underlying these conditions are not identical.

Focus upon external or internal causations for morbidity and mortality must indeed be managed with caution, notably since sweeping yet unwarranted conclusions are commonplace. Thus, snow shoveling in Minnesota has been blamed without qualification for 'heart attacks' and consequent deaths in the winter months.

Data in fig. 13 reveal a similar timing for a circannual mortality rhythm from heart disease in different regions of the United States, some of them being areas with minimal or no snowfall. Actually data in fig. 2 show that morbidity and mortality from heart and respiratory disease, on a world-wide scale, are non-randomly distributed. Before premature conclusions are drawn as to underlying factors, much work remains to be done in the laboratory and in the field, to examine the factors underlying rhythmic characteristics of populations.

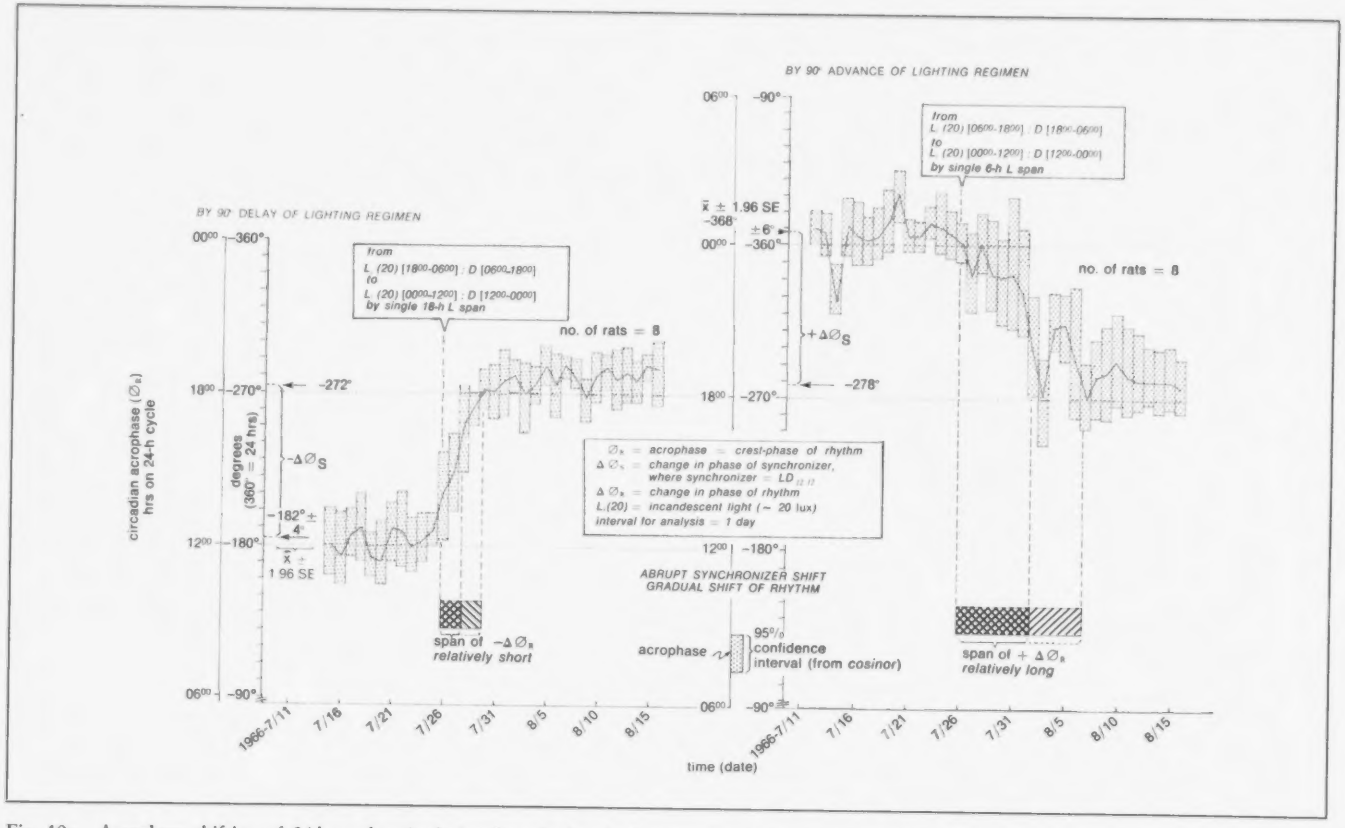


Fig. 10a - Acrophase shifting of 24-h-synchronized circadian rhythm in intraperitoneal temperature of female MSD rats.

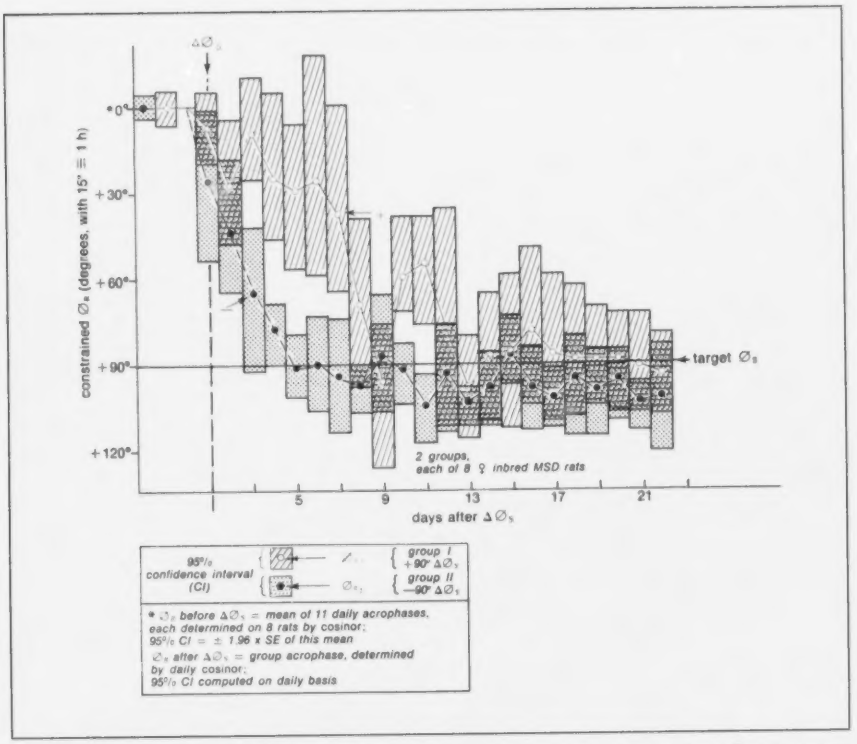

constrained ∅R (degrees, with 15° ≡ 1 h)
0°
+30°
+60°
+90°
+120°
target ∅R
2 groups,
each of 8 ♀ inbred MSD rats
5
9
13
17
21
days after Δ∅s
95% confidence interval (CI)
group I
+90° Δ∅s
group II
—90° Δ∅s
* ∅R before Δ∅s = mean of 11 daily acrophases, each determined on 8 rats by cosinor; 95% CI = ± 1.96 x SE of this mean
∅R after Δ∅s = group acrophase, determined by daily cosinor; 95% CI computed on daily basis

Fig. 10b - Difference in shift-behavior of circadian acrophase, $\varnothing_R$, for intraperitoneal temperature after a 90°-advance (+) or delay (—) of the synchronizing regimen of light and darkness alternating at 12-h intervals: $\Delta\varnothing_s$ ($LD_{12:12}$).

Recent studies have focused primarily upon the importance of the hours of changing resistance, i.e. upon susceptibility rhythms with a period of about a day, rather than a week, month or year.

Conceivably, peaks in morbidity or mortality from different diseases that recur more frequently at certain times during a given cycle do not represent the result exclusively of a single environmental factor impinging on us at a clock hour, time of week or time of year. Quite likely, different time relations among rhythms are encountered by extrinsic factors in different stages of a given rhythm. Against this background it is desirable, if not mandatory, to consider the organism's rhythmic structure in the design of clinical or other tests measuring responses, adjustability or vulnerability. Glucose tolerance tests are but one case in point [7, 20, 21, 55]. Before timed tests of, say, a pancreatic and/or pituitary hormonal system become meaningful, underlying mechanisms must be analyzed. In this way, eventually, we may become able to identify changes in the organism's physiologic or pathologic state that allow us to anticipate cyclic challenges and to cope with them successfully.

While rhythms in adjustability and vulnerability must not be ignored by those employing tests based upon rhythmic endpoints in order to screen for risk or diagnose disease, these same response rhythms may be exploited more directly to define health. Moreover, the student of chronobiology defines health not only and not even pri-

marily in terms of vulnerability. It seems to be equally promising to direct attention toward spontaneous change, whenever one formulates a positive personalized definition of health in psycho-physiological terms. The affluent technologically developed stratum of our society may argue that man's actual environment, apart from his geophysical one, can be kept rather constant. Heating, air-conditioning, lighting and air-freighting of most kinds of foods in all seasons can compensate for natural extremes in temperature, illumination and food supply within our *habitat* niche. One may therefore raise the question whether constancy may not be more desirable than periodicity for man's body functions, even under environmental conditions rendered as time-invariant as possible.

Such a line of thought recognizes that almost certainly rhythms represent an evolutionary adaptation to cyclic pressures of the environment [3, 16, 22, 54, 72]. However, such assumptions ignore another principal aspect of our rhythmic time structure, namely the possibility that, apart from any environmental adjustments, rhythms are required for a successful intrinsic temporal integration of body functions. It seems likely that once evolved and genetically anchored, the multitude of rhythms, constituting a spectrum of frequencies and exhibiting a particular timing at each frequency, represents a sophisticated temporal network — a *consensus partium in tempore* [22].

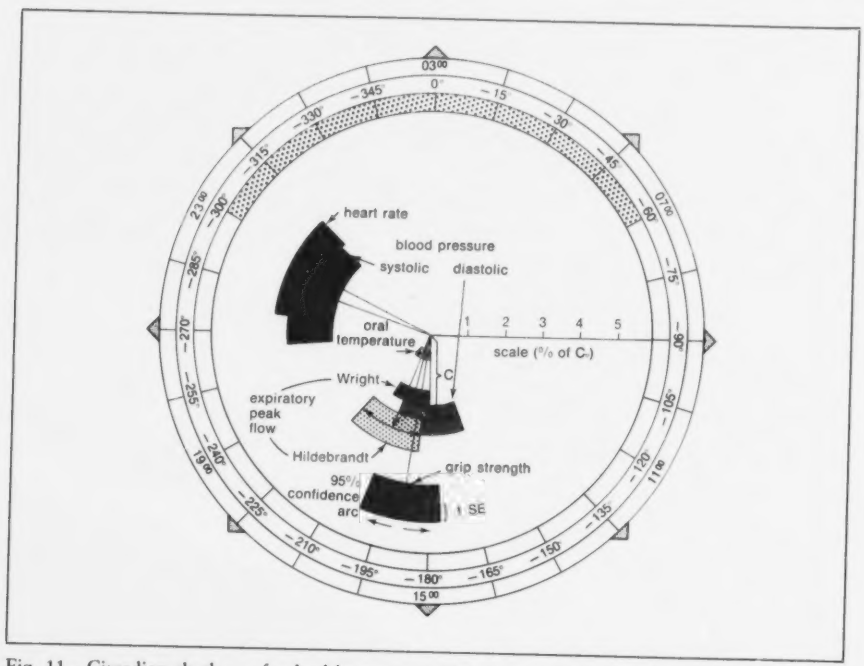


Fig. 11 - Circadian rhythms of a healthy woman. Circadian amplitudes and acrophases: the data were obtained by autorhythmometry. Measurements were made several times a day only during the activity period (no. ~ 750 during 17 months). The dotted area represents the sleep span, the acrophase is indicated by the arrow direction and the amplitude by the arrow length. The amplitudes are expressed as the % of M. Temperature is measured in Celsius degrees. The transformation of temperature in % of M is not justified: absolute values may be used for this variable. Circadian rhythms of oral temperature, grip strength, diastolic blood pressure and peak expiratory flow (evaluated in two ways) are correlated. The self-measurements of the peak expiratory flow and grip strength may be used as guides for the chronotherapy of asthma and *myasthenia gravis*.

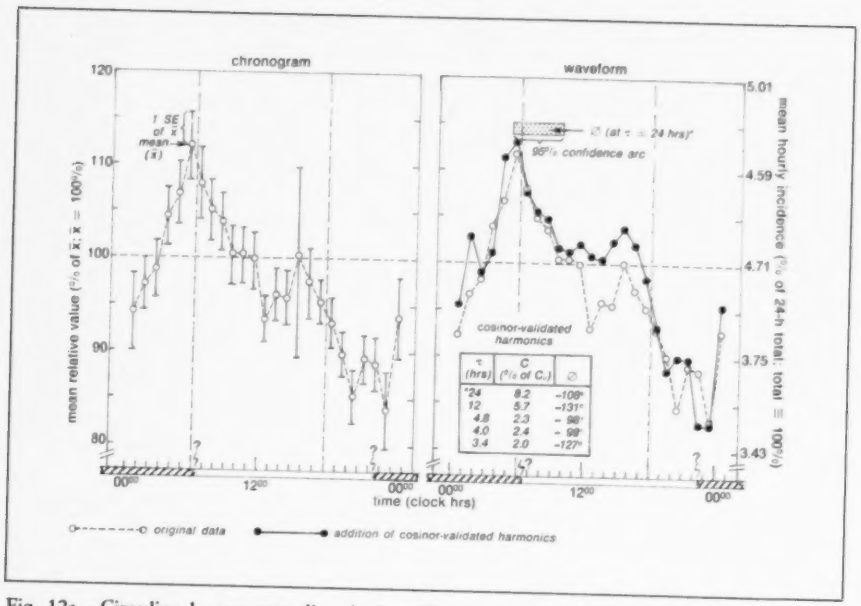


Fig. 12a - Circadian human mortality rhythm. The occurrence of human death along the scale of 24 hrs suggests circadian rhythmicity with greatest incidence around 06[30]. Irregularities, e.g. secondary crests, may reflect different causes of death, ultradian frequencies and/or synchronization schedules in samples prior to death. Comparison of the circadian waveform (right-hand figure), constructed from statistically significant periodicities validated by *cosinor*, with original data is good. Note: shaded regions at bottom of figures approximate assumed nocturnal rest spans.

timing in initial week	subjects		weight change (+ = gain; — = loss)		
	sex	age	breakfast only (lbs./week)	dinner only (lbs./week)	difference (lbs.)
D	M	39	—3.81	+1.18	4.99
		24	—1.85	—0.37	1.474
		21	—2.22	+0.37	2.596
	F	19	—1.85 *	+1.11	2.948
		18	—2.22 *	+0.37	2.596
B	M	19	—2.22	+1.11	3.322
		19	—4.07	—1.48	2.596
pool			—2.60	—0.33	2.93 *

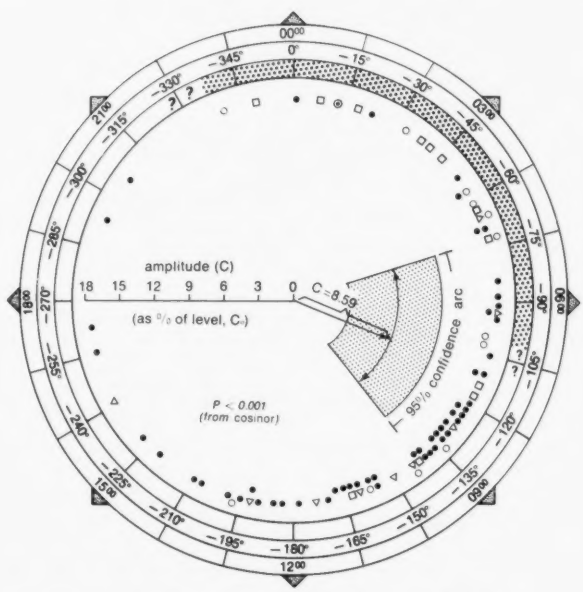
* $P < 0.001$.

Table 3 - Body weight change during 1 week on a single daily meal consumed as breakfast (B) or dinner (D).

Clinical research should therefore include chronobiological analyses in order to examine the possibility that alterations in this network can become determinants of disease. Modifications of circadian rhythms already have been encountered in several specific conditions to which organisms may be exposed. The question to be solved is the extent, if any, to which such alterations, or others, determine or contribute to performance decrement (fig. 14) or illness [29, 36, 46] in 1) transmeridian dyschronism; 2) intermittent catatonia; 3) emotional depression (fig. 15); 4) rheumatoid arthritis; 5) cancer or 6) peptic ulcer [10, 64, 90], to cite but a few examples. For an approach to such questions of chronohygiene and possible chronopathology, and perhaps consequent rhythm exploitation in design of life style or therapy, a review of chronobiologic methods is essential.

Time scales, rhythmometry and autorhythmometry (AR)

Before the advent of objective rhythm assessment, indications of duration in biology and medicine were given exclusively in conventional time markers and units,



mortality category	no. of cases	no. of (C, Ø)	symbols for acrophases
pediatric	21,673	12	○
post-surgical	500	1	◉
cardiovascular	7,644	9	△
pulmonary	9,357	12	□
cause unspecified	393,718	54	•
cosinor of above	432,892	88	

Fig. 12b - Circadian rhythm of susceptibility to 'death' in human beings. Polar plot summarizes the timing of individual acrophases for 88 time series representing nearly 433,000 deaths. Almost 65 % of the Ø occur between 04⁰⁰ and 11⁰⁰. Greatest overall sample mortality occurs at 07⁰⁰. Inner shaded portion indicates assumed nocturnal rest span of sample prior to death.

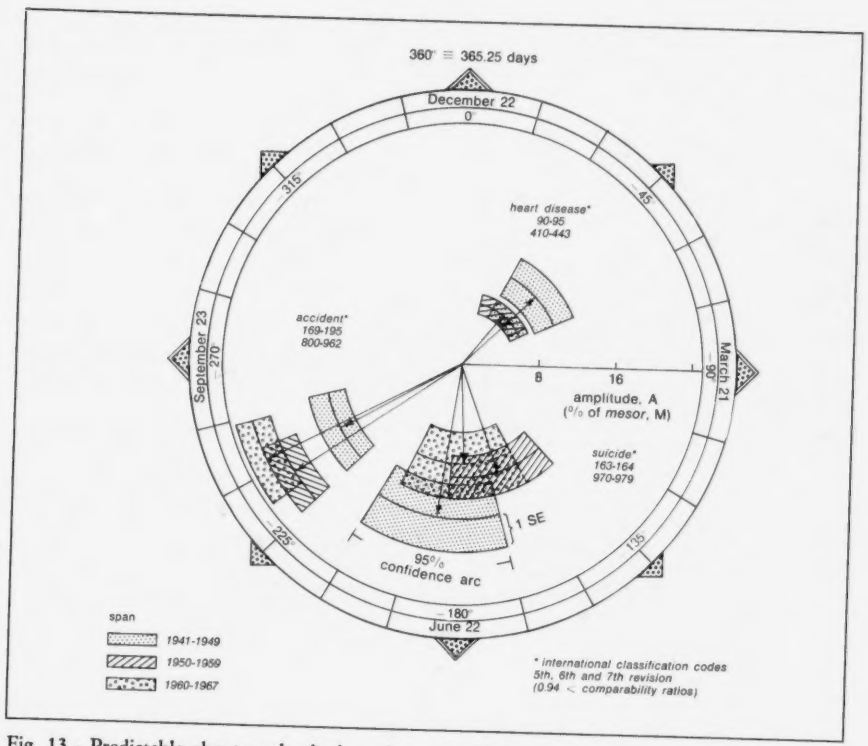


Fig. 13 - Predictable about-yearly rhythms determine likelihood of Minnesotans to escape death from heart disease, suicide or accident. Circannual rhythms of mortality in Minnesota during 1941-1967. Note reproducibility of findings in consecutive decades.

such as dates, clock hrs and min, without further qualification. This practice presumes an identical flow of biological and clock calendar time. The fallacy of this assumption emerges from several lines of evidence. Menstrual cycle lengths vary in conventional time units but these chronologically different periods may be biologically equivalent. The timing of a circadian rhythm in 17-hydroxycorticoid excretion by Europeans and Amerindians differs when it is referred to clock time but is the same when it is referred to midrest, fig. 16 [47].

Rhythmometry which assesses timing, among other characteristics, fig. 6, involves the collection of time series, the objective quantification of their temporal characteristics, and special displays that can be prepared by electronic computation.

Autorhythmometry, briefly AR, is the procedure of self-measurement followed by computer-aided rhythm analysis and self-checking and partial interpretation of results. AR serves for monitoring health with data on physiological functions and physical and mental performance. AR for a chronobiologic approach to a number of medical, social and ecologic goals requires several steps:

1) education for self-measurement,

2) instrumentation for self-measurement,

3) a strict definition and standardization of conditions of observation and measurement,

4) execution of measurements according to a scheme of sampling times and analytical steps adjusted to individual exigencies, in terms of schedules and any special health care needs prompting the individual to carry out AR,

5) means for data transfer into an analyzable form or medium, such as punched cards or magnetic tapes if not tabulations,

6) specification of a format necessitated by procedures for numerical analysis such as programs for electronic computation,

7) methods for isolation, presentation and interpretation of rhythm characteristics, including waveform, in an easily understandable useful form,

8) as part of an individualized health card (or passport) [35, 84].

With respect to the conditions of observation on ambulatory subjects, one may standardize the timing of arising and retiring in terms of a fixed clock hour for 1 week prior to the start of measurement and during the observation span. While leaving other aspects of one's ordinary schedule unchanged, in *direct* autorhythmometry, assessing behavior under habitual conditions, one may refrain from particularly unusual activities. By contrast, in the *indirect* method, one and the same test may be systematically repeated at different circadian times, and the outcomes recorded and analyzed. Physiologic performance during and following exercise or meals constitutes an example. Body weight also can constitute a pertinent response in relation to mealtiming, tab. 3 [1, 30, 69].

In the self-assessment of rhythms, the subject's role in his own health care becomes an active one. He assumes direct responsibility for the data collected by him. His physician becomes a 'coinvestigator', playing the role of consultant, no longer directly responsible for the data collected, as is the case in a classical doctor-patient relationship. As 'coinvestigator' the physician may select special instrumentation and outline the individualized procedures for monitoring and decision making, according to

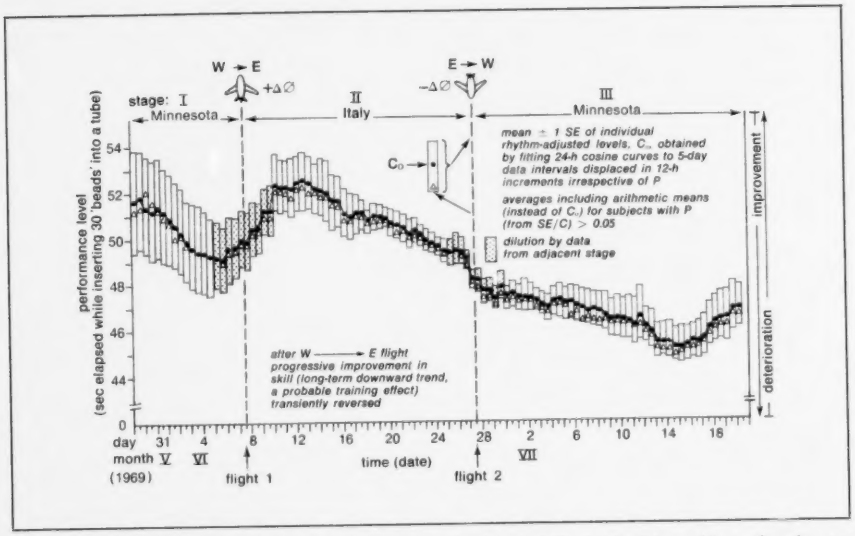


Fig. 14 - Post-transmeridian dyschronism (PTMD). Eye-hand skill of 7 healthy subjects deteriorates following West-to-East flight (advance of rhythms); similar decrement not detected after East-to-West flight (delay of rhythms).

any special needs recognized by him. However, the originally scheduled number of observations and the span covered by them may well be made dependent upon background *noise* * expected to characterize a given biologic variable investigated in a casual setting, by a teacher in high school or some other educator.

A first consideration for those concerned with autorhythmometry involves the quality of instrumentation, which determines the precision of a measurement — the resolution of an instrument scale — and its accuracy, the degree of conformity of the instrument with a national or international standard [40]. However, in the self-measurement of rhythms, the quality of instrumentation is not a sufficient condition in itself; when serial observations are being made and changes over time are the focus of the experiment, the reproducibility of the measurement techniques becomes critical. Thus, the self-measuring individual must maintain a responsible, interested attitude in securing a comparable caliber for the number of observations in time. He must maintain both the caliber of his procedures and that of his tools.

A definite effort is needed to learn all requirements of autorhythmometry through direct instruction in a special introductory teaching session and in follow-up encounters for summarizing the results. The data collected are recorded on a flowchart for chronobiological sampling (fig. 17) [40]. These data may consist of a few physiological variables, such as blood pressure, core temperature, pulse, grip strength, adding speed, finger counting and *tempo* **. In follow-up sessions, the schedule and the kinds of measurements may be modified according to specific aims.

Autorhythmometry and the usual range

AR places responsibility upon the individual not only for the correct collection,

* *Noise* is the effect caused by various occasional agents that may interfere with the signal (e.g. a rhythm) and thus with a correct data registration or evaluation.

** *Tempo*: the subject measures (with a chronometer) the time span necessary to count, for example, from 1 to 120, to evaluate a 2-min period.

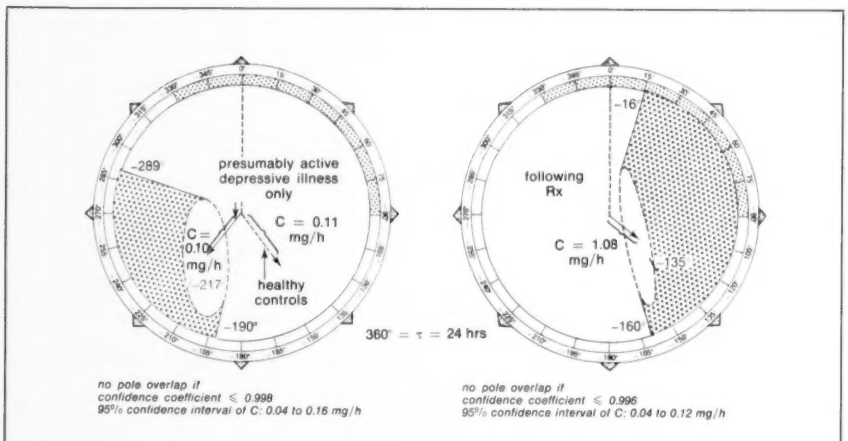


Fig. 15 - Circadian rhythm of 17-ketosteroid excretion evaluated by *cosinor*. Patients with depressive illness. Original data of SAKAI M.: Yokohama med. Bull. *11*, 352, 1960. Synchronizer schedule extrapolated from timing of urine collection spans.

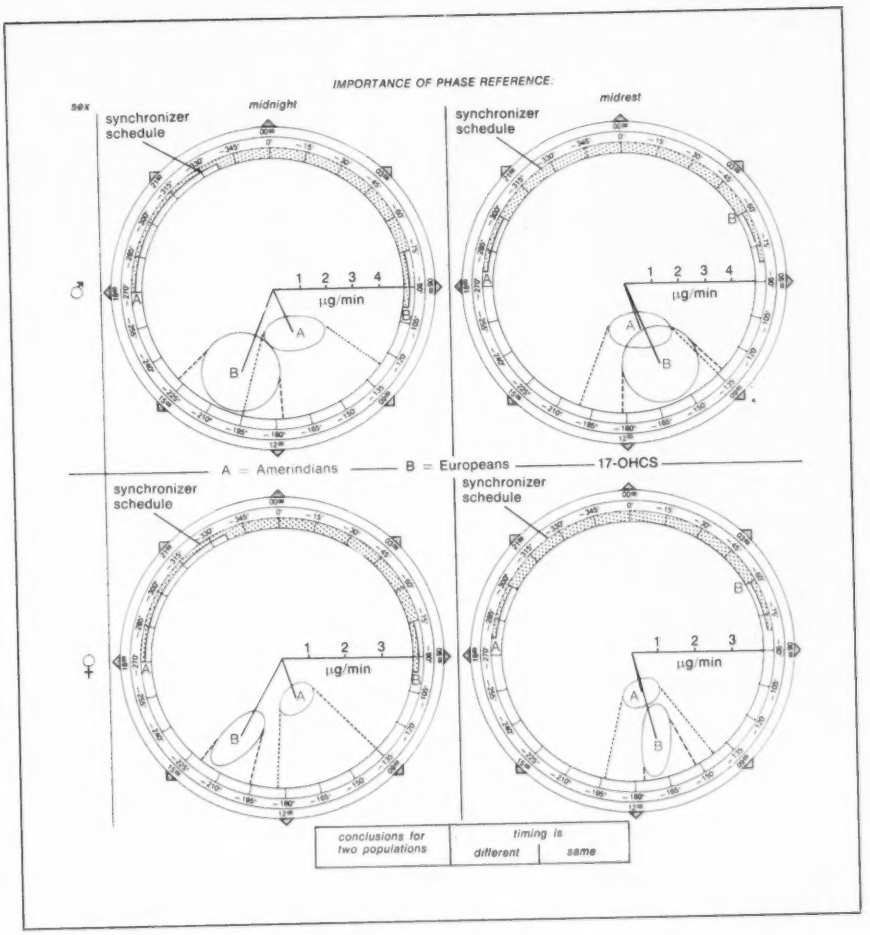


Fig. 16 - Timing of circadian rhythms in 17-hydroxycorticoid excretion by Europeans and Amerindians differs when it is referred to clock time (left); yet it is the same when it is referred to mid-rest (right). In the *cosinor* display in the left column, the computative acrophase, Ø, is the angle formed by a directed line with the reference time at midnight, shown as zero degrees. The amplitude, A, is shown by the length of a directed line. Values for Ø and A are given in the *cosinor* displays. The figure reveals a statistically significant rhythm for both the Amerindians and the Europeans. When midnight is used as reference time, the difference in acrophase is statistically significant for women in view of the non-overlapping 95 % confidence arcs. When the reference time is changed to the middle of the habitual sleep span (right), the internal acrophases no longer differ. Thus, a simple, common-sense adjustment in reference time eliminates a spurious intergroup difference and provides an objective, quantitative, gross-culturally valid index for the timing of an important rhythm in different populations.

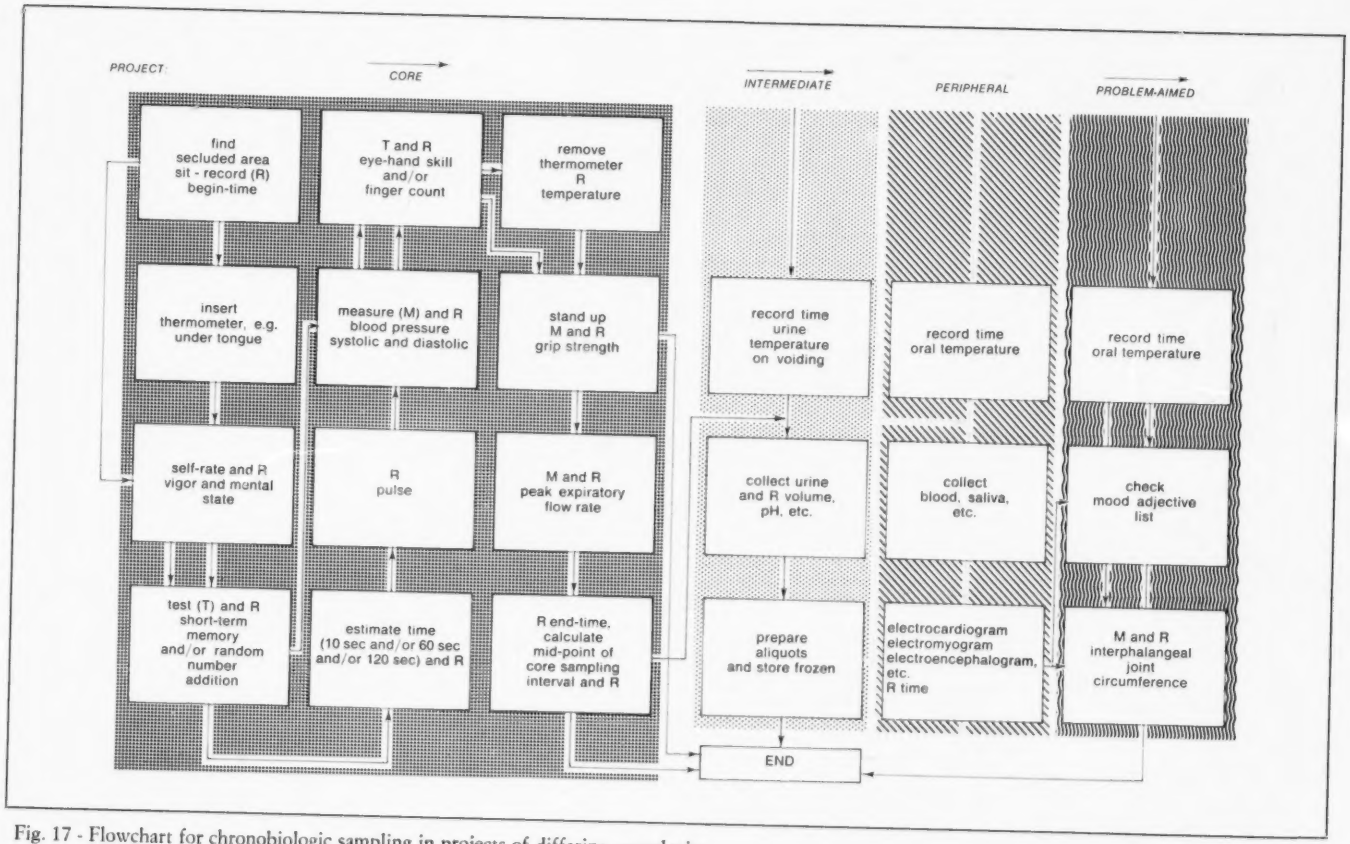

PROJECT
CORE
INTERMEDIATE
PERIPHERAL
PROBLEM-AIMED
find secluded area sit - record (R) begin-time
T and R eye-hand skill and/or finger count
remove thermometer R temperature
insert thermometer, e.g. under tongue
measure (M) and R blood pressure systolic and diastolic
stand up M and R grip strength
self-rate and R vigor and mental state
R pulse
M and R peak expiratory flow rate
test (T) and R short-term memory and/or random number addition
estimate time (10 sec and/or 60 sec and/or 120 sec) and R
R end-time, calculate mid-point of core sampling interval and R
record time urine temperature on voiding
collect urine and R volume, pH, etc.
prepare aliquots and store frozen
record time oral temperature
collect blood, saliva, etc.
electrocardiogram electromyogram electroencephalogram, etc. R time
record time oral temperature
check mood adjective list
M and R interphalangeal joint circumference
END

Fig. 17 - Flowchart for chronobiologic sampling in projects of differing complexity.

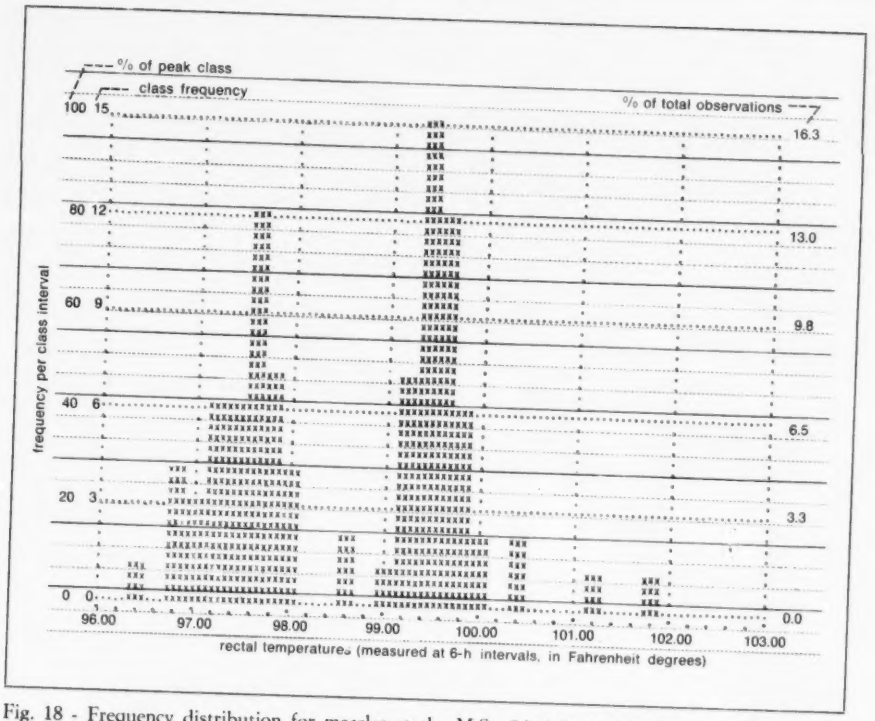


Fig. 18 - Frequency distribution for measles study. M.S., 5/1/1961-5/23/1961. No. = 92; class interval = 0.40; mean = 98.54; SD = 1.16. Histogram of rectal temperatures measured at 6-h intervals for 23 days on a subject with a prominent circadian rhythm in this variable. Note that bimodality, rather than a bell-shape, characterizes the frequency distribution of the temperatures.

verification and transfer of data to a computer but also for the handling if not the interpretation of computer analysis of rhythms; to those having motivation as well as the education shared by many individuals in developed areas, AR makes available at minimal cost certain otherwise unattainable features of health care.

Repeated measurements made in seeming health over appropriate spans of time provide an individually pertinent data base, whether or not a portion of the overall variability can be distinguished as being predictable (e.g. rhythmic) rather than random. Indeed, the extent of change in one's physiologic and psychologic functions, such as core temperature and blood pressure, is of interest for assessing performance and the state of one's health. By comparison, when an illness is already present, the opportunity to collect an unquestionably healthy reference standard for the same individual may be irretrievably lost. But even if the individual is 'healthy', only limited information about him is gained from the conventional single measurement made in a physiology laboratory or by a physician, and all of this is often promptly lost again, when the 'healthy' individual is dismissed from a practitioner's office because his vital signs, for instance, are 'within the usual range of a sex and age comparable cohort'.

Under such conditions many clinical measurements indeed become so meaningless for the individual that they are justifiably discarded. Whether or not we can get

refined information from as conventional a procedure as thermometry will depend on whether we 1) simply anticipate that the given single body temperature measurement is above or below an imaginary straight line that we draw between health and disease, or 2) make use of rhythmometry.

Fig. 18 shows that the usual line drawn on the clinical thermometer at 37 °C. may be misleading. Few values in a large sample of repeated measurements on one and the same presumably healthy individual correspond to the 'normal' temperature. The sample of temperature values, as a whole, clusters almost symmetrically around both sides of that arbitrary value. But even after limits are drawn with regard for rhythms, the definition of fever as a *pathologically* high temperature must be scrutinized further.

In practice it does not suffice to find a temperature elevation. Before using this finding in the diagnosis of a disease, one has to rule out the presence of undefined 'health'. Otherwise, the temperature elevation can be labeled as *hyperthermia* rather than *fever*. For the latter purpose, the practitioner of medicine uses usually several other signs or symptoms and these too may well be rhythmic! Equally important therefore seems to be the need to evaluate for diagnosis alterations in all of the measurable and pertinent characteristics of all the relevant rhythmic variables — e.g. deviations from a rhythm-adjusted mean (rather than from imaginary horizontal lines or ranges) and/or alterations in a spectrum of rhythms that can now be resolved concomitantly for several variables.

We take pride, for instance, in reading a body temperature to the nearest decimal, say, in degrees Celsius, while we regard the spectrum of its rhythms, fig. 19, as somewhat esoteric. Quite a few authors utilize for natural family planning a 'basal' temperature reading [18], representing the interaction of a circadian, a circatrigintan (or

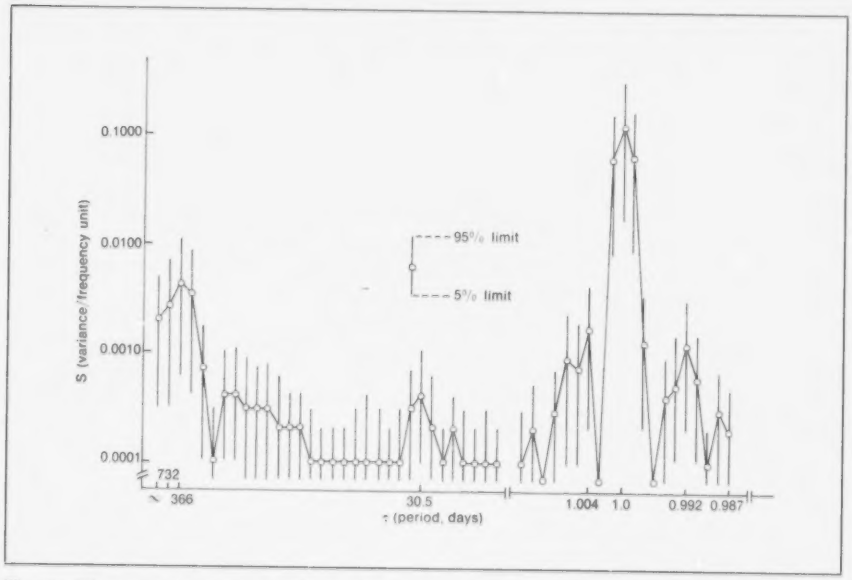


Fig. 19 - Thermovariance spectrum of rectal temperature. D.W., ♂, born on 3/3/1944. No. = 2,704; m = 1,464; Δt = 0.25 days; variance = 0.45; circadian quotient (CQ) = 65 % from 7/13/1960 to 5/19/1962.

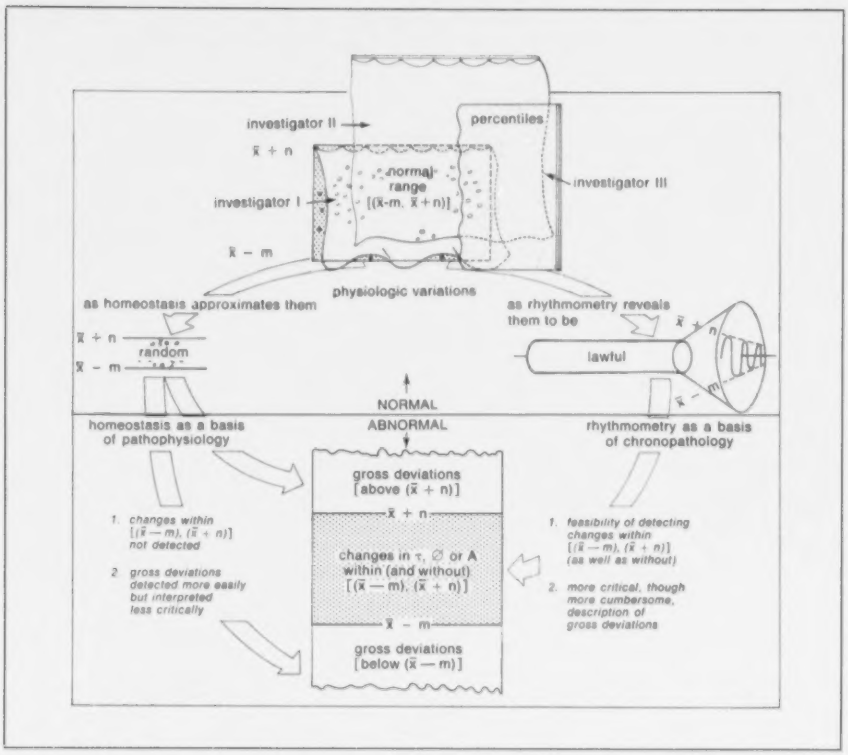

investigator II
percentiles
$\bar{x}$ + n
normal range
[($\bar{x}$-m, $\bar{x}$+n)]
investigator I
investigator III
$\bar{x}$ − m
physiologic variations
as homeostasis approximates them
as rhythmometry reveals them to be
$\bar{x}$ + n
random
$\bar{x}$ − m
lawful
NORMAL
homeostasis as a basis of pathophysiology
ABNORMAL
rhythmometry as a basis of chronopathology
gross deviations [above ($\bar{x}$ + n)]
$\bar{x}$ + n
changes in τ, ∅ or A within (and without) [($\bar{x}$ − m), ($\bar{x}$ + n)]
$\bar{x}$ − m
gross deviations [below ($\bar{x}$ − m)]
1. changes within [($\bar{x}$ − m), ($\bar{x}$ + n)] not detected
2. gross deviations detected more easily but interpreted less critically
1. feasibility of detecting changes within [($\bar{x}$ − m), ($\bar{x}$ + n)] (as well as without)
2. more critical, though more cumbersome, description of gross deviations

Fig. 20 - *On the left*: the fundamental error of contemporary biology (homeostasis) leads to neglect of physiologic variability regarded tacitly as negligible if not random. *On the right*: rhythmometry enables insight into characteristics of predictable biologic variability.

menstrual) and a circannual component. The modulations (by the aforementioned lower-frequency rhythms) of the circadian one involve measureable parameters. Changes in the latter may help to render methods of family planning more reliable and more generally applicable. The availability of appropriate instrumentation both for the automatic and frequent recording of temperatures and for assessing their rhythm spectrum eventually will have to complement AR in school and beyond, as well as in the clinic. In experimental animals, telemetry already has served to assess rhythmic temperature behavior over most of their lifetime. The student of aging may be interested in changes of circadian rhythm characteristics, such as amplitude, already known to characterize aging in mammals [17, 32, 93].

To turn back to human temperature, if autorhythmometry or automatic rhythmometry should yield appropriate time series, one could examine any changes in rhythm characteristics that precede the occurrence of fever or take place within the physiologic range in the absence of fever. Indeed as fig. 20 visualizes, the promise of rhythm analysis — by self-measurement, physician-assessment and/or automatic means — lies in its ability to examine any changes in human variability within the precise range in which this variability usually occurs. It is from this viewpoint that autorhythmometry and its implications for health care may be examined with particular reference

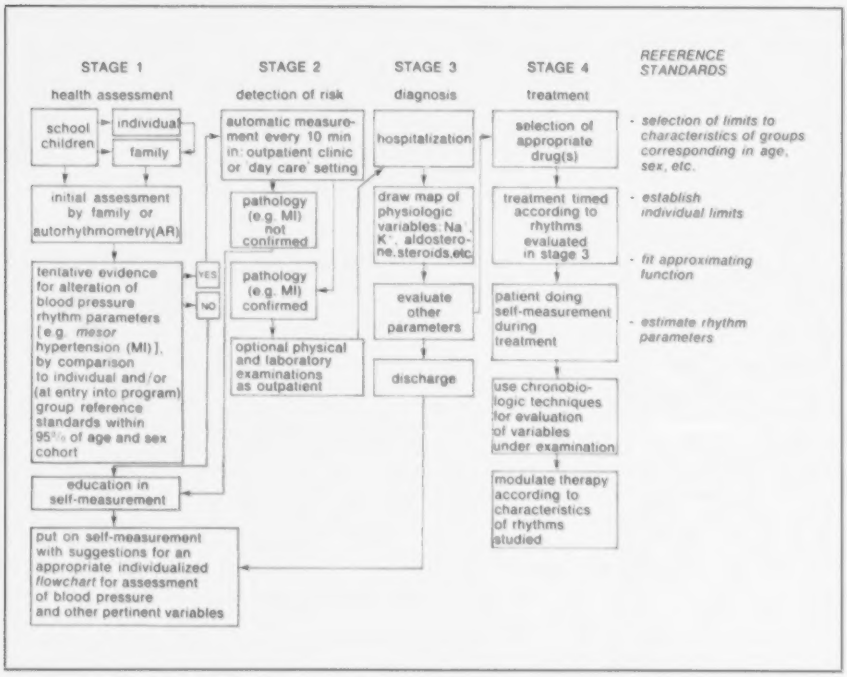
to a variety of functions, such as temperature, heart rate, the characteristics of the ECG and EEG and, last but not least, with respect to blood pressure.

Autorhythmometry of blood pressure

Hypertension is the major debilitator and killer in many countries; yet often the condition can be corrected efficiently. The earlier that high blood pressure, or the risk of its occurrence, is detected and treated, the less the amount of irreversible damage done. Given sufficient properly scheduled measurements, computer methods can determine the predictable aspects of patterns in pressure elevation over time, detecting, *inter alia*, 'odd-hour-hypertension' or other forms of labile or fixed pressure elevation. Moreover, even in health, the definition of individualized usual ranges is important. This point is illustrated in fig. 21: during the resolution of a post-streptococcal glomerulonephritis with hematuria, as well as after full recovery, a 4-year-old girl has diastolic blood pressures that lie within the 'usual range' for the corresponding sex and age group [37]. After the nephritis resolves, the hematuria subsides, and the pressure drops. When compared to this child's own pressures after recovery the pressure during nephritis had been clearly elevated.

Hence, reliance upon the range of a cohort, as is currently the practice, may be complemented by interpretations based upon comparisons with individualized (rhythm-adjusted) means, *mesors* or other rhythm characteristics.

Whether or not available data suffice for rhythm definition, the provision of a personalized usual range for the given individual constitutes an invaluable dividend


STAGE 1
health assessment
STAGE 2
detection of risk
STAGE 3
diagnosis
STAGE 4
treatment
REFERENCE STANDARDS
school children
individual
family
initial assessment by family or autorhythmometry(AR)
tentative evidence for alteration of blood pressure rhythm parameters [e.g. mesor hypertension (MI)], by comparison to individual and/or (at entry into program) group reference standards within 95% of age and sex cohort
YES
NO
education in self-measurement
put on self-measurement with suggestions for an appropriate individualized flowchart for assessment of blood pressure and other pertinent variables
automatic measurement every 10 min in 'outpatient clinic or 'day care' setting
pathology (e.g. MI) not confirmed
pathology (e.g. MI) confirmed
optional physical and laboratory examinations as outpatient
hospitalization
draw map of physiologic variables: Na+, K+, aldosterone, steroids, etc.
evaluate other parameters
discharge
selection of appropriate drug(s)
treatment timed according to rhythms evaluated in stage 3
patient doing self-measurement during treatment
use chronobiologic techniques for evaluation of variables under examination
modulate therapy according to characteristics of rhythms studied
- selection of limits to characteristics of groups corresponding in age, sex, etc.
- establish individual limits
- fit approximating function
- estimate rhythm parameters

Scheme 1 - Chronobiology and chronopathology of blood pressure.

entry no.	criterion syst/diast	references
		'hypotension' and 'normotension'
1	110/—	MacNulty A. S. (Ed.): British Medical Dictionary - J. B. Lippincott Co., Philadelphia, 1963; p. 721
2	110/66 (male) 105/62 (female)	Dally M. E., Halls J. F.: Low Blood Pressure. Its Causes and Significance - William Wood and Co., New York, 1928; pp. 27 and 31
		'normotension' and 'hypertension'
3	—/95	Puddu V.: Cardiovascular Regulation in Health and Disease - In: 1st International Symposium of the Cardiovascular Research Institute. Milano, 1970; p. 212
4	120/80	Robinson S. C., Brucer M.: Range of Normal Blood Pressure. A Statistical and Clinical Study of 11,383 Persons - Arch. intern. Med. *64*, 409, 1939
5	130/70	Browne F. J.: Chronic Hypertension and Pregnancy - Brit. med. J. *2*, 283, 1947
6	140/80	Ayman D.: Heredity in Arteriolar (Essential) Hypertension: A Clinical Study of the Blood Pressure of 1,524 Members of 277 Families - Arch. intern. Med. *53*, 792, 1934
7	140/90	Reid D. E., Teel H. M.: Nonconvulsive Pregnancy Toxemias - Amer. J. Obstet. Gynec. *37*, 886, 1939
8	140/90	Chesley L., Annitto J.: Pregnancy in the Patient with Hypertensive Disease - Amer. J. Obstet. Gynec. *53*, 372, 1947
9	140/90	Perera G. A.: Diagnosis and Natural History of Hypertensive Vascular Disease - Amer. J. Med. *4*, 416, 1948
10	140/90	Schroeder H. A.: Hypertensive Diseases. Causes and Control - Lea and Febiger, Philadelphia, 1953; p. 29
11	140/90	Mayer J. H., Flynn J.: The Role of Arterial Hypertension in Coronary Atherosclerosis - In: Russek H. I., Zohman B. L. (Eds): Coronary Heart Diseases. J. B. Lippincott Co., Philadelphia, 1971; p. 138
12	150/90	Thomas C. B.: The Heritage of Hypertension - Amer. J. med. Sci. *224*, 367, 1952
13	150/90	Hoobler S. W.: Hypertensive Disease. Diagnosis and Treatments - Paul B. Hoeber, New York, 1959; p. 70
14	150/90	Bartorelli C.: Essential Hypertension - Springer-Verlag, Berlin, 1960; p. 28
15	150/95	Moser M., Goldman A. G.: Hypertensive Vascular Disease. Diagnosis and Treatment - J. B. Lippincott Co., Philadelphia, 1967; p. 11
16	150/100	Ask-Upmark E.: A Primer of High Blood Pressure - Svenska Bokförlget, Bonniers, 1967; p. 14
17	160/100	Bechgaard P.: Arterial Hypertension: A Follow-Up Study of One Thousand Hypertonics - Acta med. scand. (Suppl. 172), 1946
18	180/100	Burgess A. M.: Excessive Hypertension of Long Duration - New Engl. J. Med. *239*, 75, 1948
19	180/100	Evans W.: Cardiology - Butterworths, London, 1956; 2nd Edition, p. 386
20	from 140/90 to 150/100	Smirk F. H.: High Arterial Pressure - Blackwell Scientific Publ., Oxford, 1957; p. 18
21	90/63 (3 years) 115/75 (16 years) 140/90 (40 years) 150/90 (over 40)	Goldring W., Chasis H.: Hypertension and Hypertensive Disease - The Commonwealth Fund, New York, 1944; p. 9

Table 4 - Dividing lines among 'hypotension', 'normotension' and 'hypertension'. Amplified on: Pickering [73], tab. 3.1.

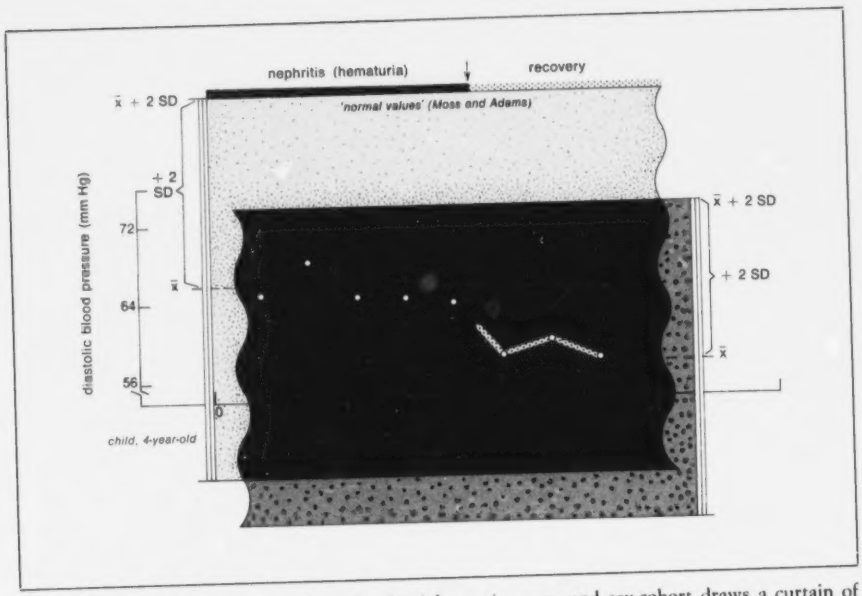


Fig. 21 - Exclusive reliance upon 'usual values' for a given age and sex-cohort draws a curtain of ignorance over the range of physiological variation in health, states of risk or overt diseases. This curtain can be lifted by rhythmometry; thus one detects (within the 'normal range' of a peer cohort) 'individualized' *mesor*-hypertension (a statistically significant elevation in the rhythm-adjusted mean of blood pressure assessed in relation to an individualized *mesor*). Higher blood pressure during nephritis (left), than after recovery (right). Yet all values are within range generally considered normal.

of autorhythmometry. Fig. 22 shows a lack of overlap for the '100 % ranges' of several presumably healthy girls and boys. If individual ranges differ, the overall cohort range (even with corrections for sex, age and other aspects of an individual's background) is anticipated to be wide. This width renders rather difficult any attempt to distinguish between blood pressures in health and in disease. Past endeavors to 'draw a line' have been criticized by PICKERING [71], who has pointed to the discrepant literature. His line of thought is slightly amplified in tab. 4. Obviously, no dividing line can be agreed upon on the basis of 'standards' from time-unqualified spot-checks. Alternatively, whenever possible, a judgement concerning the normality or abnormality of an individual's blood pressure should be subjected to scrutiny by accounting for variability. As early as 1894, KIESOW [57] and COLOMBO [14], both working in Alfredo Mosso's laboratory in Torino, had recognized changes in a (then measured mean) human blood pressure. Fig. 23 presents Colombo's original data describing spontaneous variations of blood pressure in a healthy man, while fig. 24 summarizes some of Colombo's data (left-hand section of fig. 23) by a single *cosinor* [40], revealing statistically significant differences in timing among circadian rhythms in four body functions.

Of particular interest is Colombo's comment in 1899 that autonomic variations in blood pressure and in closely related factors, such as heart and respiratory rates and body temperature, are atavistic and hereditary. He writes that they are not the immediate consequence of different states of work and rest, of starvation and of external meteorologic conditions. Of further interest in fig. 23 are observations on a reportedly post-prandial decrease in blood pressure, although Colombo's text reveals that

the meals consumed by him included wine; apparently no separation of the effects of food and drink was attempted. Today, such a study would focus upon circadian time-dependence of the various agents to be investigated, since for alcoholic beverages [74, 81, 91] as well as for food [44, 69, 83] and drug [30, 38, 77, 78, 79, 80], a circadian time-dependence of effects has been established.

To turn back to self-measurements: not only can these provide more extensive data than the single measurements usually collected in an office by a physician or other health care personnel, but the measurements can more readily be made under varied conditions of ordinary life by automatic rhythmometry [40]. Moreover, such measurements may be compared in terms of rhythm characteristics, notably *mesors* by *mesor*-tests. In addition, any differences in the amplitude and/or acrophase can now be determined by a combined amplitude and acrophase test [67]. Rhythm characteristics as standards obtained by a combination of self-measurements and automatic measurements should add greatly to the efficiency of our preventive measures as well as to screening, diagnosis and therapy.

Once a statistically significant *mesor* elevation is detected and treatment instituted, we again can rely on autorhythmometry to follow the effect of treatment. One then designs tests for optimizing individually the time-scheme of treatment with drugs, so that these are administered with least undesired effects and most desired effects [49, 51, 68, 78, 79, 80, 85, 94]. Animal experiments already completed with chlorothiazide, for example, indicate the potentialities of such timed administration.

Moreover, blood pressure autorhythmometry can be combined with self-measurements of grip strength, other physical and mental performance and even mental-state ratings: one then assesses any changes in performance or mood that may be asso-

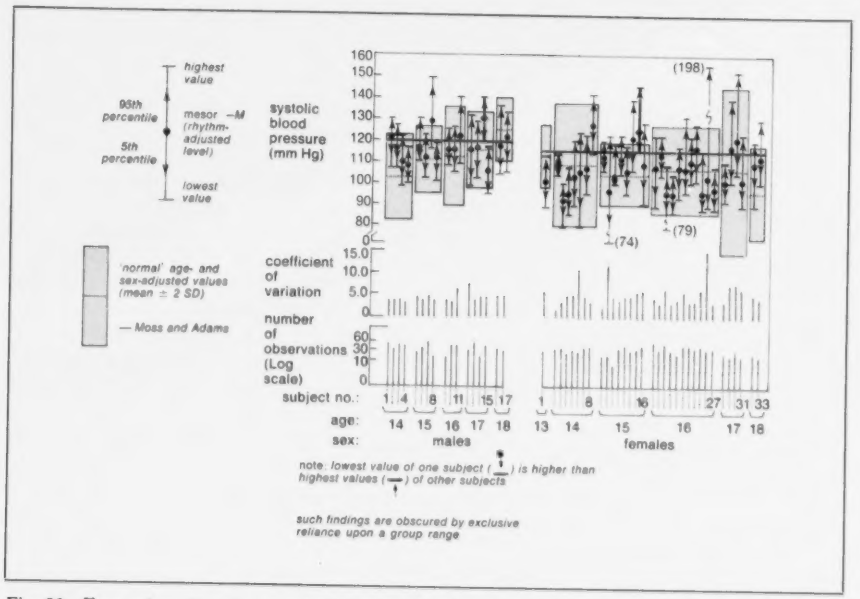


Fig. 22 - Even when data do not suffice for rhythm description, the provision of usual ranges for a given individual (rather than for a group) constitutes an invaluable dividend of autorhythmometry. It is important to know indeed that the systolic blood pressure of say, 115 mm Hg can be 'too high' for one child but 'too low' for another of the same sex and age (see text).

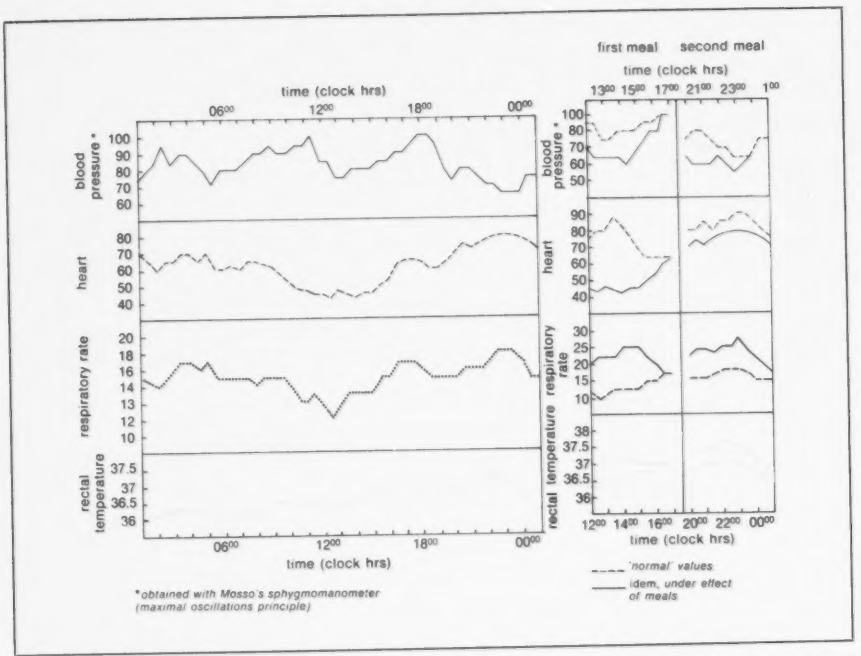


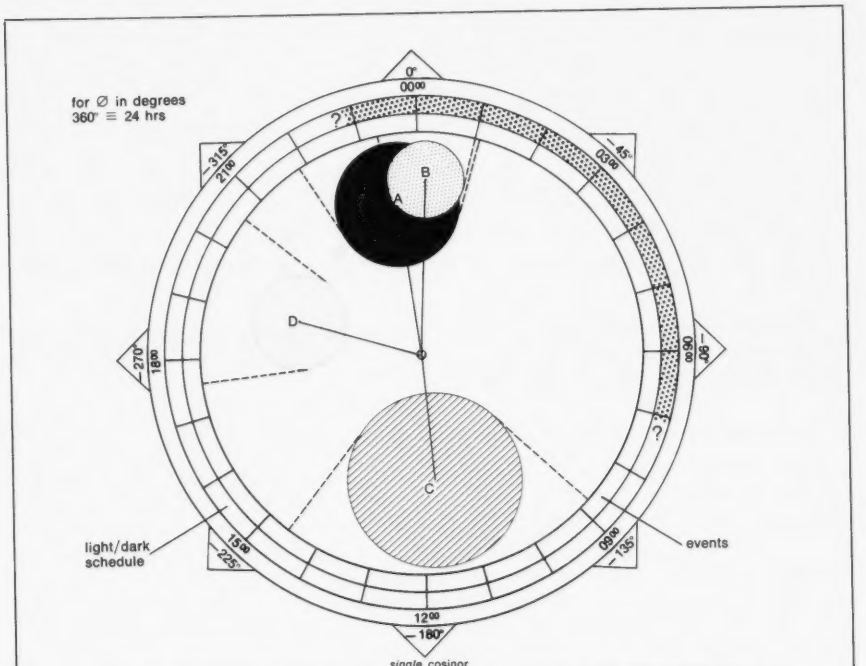
Fig. 23 - Blood pressure, heart and respiratory rates and rectal temperature in degrees Celsius of a subject weighting 67 kg, 1.68 m in height, healthy and robust, 25 years of age. (COLOMBO, 1899 [14]).

ciated with changes in blood pressure. Thus, after initiating treatment for the individual with elevated values one may search for a pressure that allows him optimal performance.

In addition to monitoring productivity in health or treatment of disease, autorhythmometric methods can also provide information that assists in pinpointing specific stages in the evolution of a certain disease not otherwise recognizable at the outset. Indeed, such methods can contribute a more solid and informative data base for information on (and the follow-up of) many diseases. It follows, then, that any body functions recognized as rhythmic must no longer be dealt with as either randomly variable or constant. At the very least, the interpretation of the single samples collected for conventional clinical (biochemical or morphologic) pathologic diagnosis should be time-qualified. Single time-point samples at a medical check-up or in an emergency should be compared with a usual range from a cohort (and for the individual, when available) that is corrected for presumably 24-h synchronized circadian rhythms and eventually for other time parameters as well. Moreover, cohort ranges will be more readily amenable to interpretation, even when they are established from single samples, if they are taken against an autorhythmometric background or at least with information regarding schedules of sleeping and waking, preferably by recording for the week before sampling rather than from recall [88].

It is practicable and economically feasible to establish for selected subjects and eventually for the school population as a whole, individualized normal ranges by way of a rhythmometric profile in health. Such information, including blood and urinary data, could become a routine part of the secondary education *curriculum*. For adults

the same AR might well constitute a routine at any hospitalization, or at 'out-patient' check-ups. Thereby, a personalized updated *status quo*, actually an 'as-one-goes' health history, would be gained, in school, without hospitalization of the individual and even without restriction of his life style. Whereas more often than not contemporary care of disease (our current practice certainly does not deserve the term health care) intervenes only after a full symptomatology has developed, i.e. in the second or subsequent acts of a given disease, rhythmometry aims first and foremost at prevention although its usefulness in diagnosis and treatment also deserves further tests. In the context of global problems, autorhythmometry can have tangible applications in a better utilization of food, drink or drugs [30] or as a basis for developing more efficient and generally accepted methods for population control [19, 31]. Assessing the parameters of human female reproductive cycles may compare favorably with a notoriously unreliable 'rhythm' method, in its many versions, which currently fails to exploit the information provided by chronobiologic techniques. Any rhythms detected by autorhythmometry in male gonadal and related neuro-humoral functions also might be utilized for control of reproduction by scheduling or other means.



key to ellipses	P	no. obs.	PR*	mesor	SE	amplitude (95% CI**)	acrophase (95% CI)
A - respiratory rate	< 0.001	49	46.5	15.0	0.19	1.71 (1.02 2.39)	—352 (—16 —328)
B - heart	< 0.001	49	74.8	61.6	0.79	12.87 (10.08 15.66)	— 2 (—15 —349)
C - blood pressure	0.002	49	23.4	83.3	1.17	6.14 (2.00 10.28)	—175 (—218 —132)
D - temperature	< 0.001	49	48.0	36.6	0.03	0.29 (0.15 0.49)	—286 (—308 —263)

*PR = rhythms (%); **CI = confidence interval

Fig. 24 - Microscopic analysis of the data from COLOMBO [14] summarized on fig. 23.

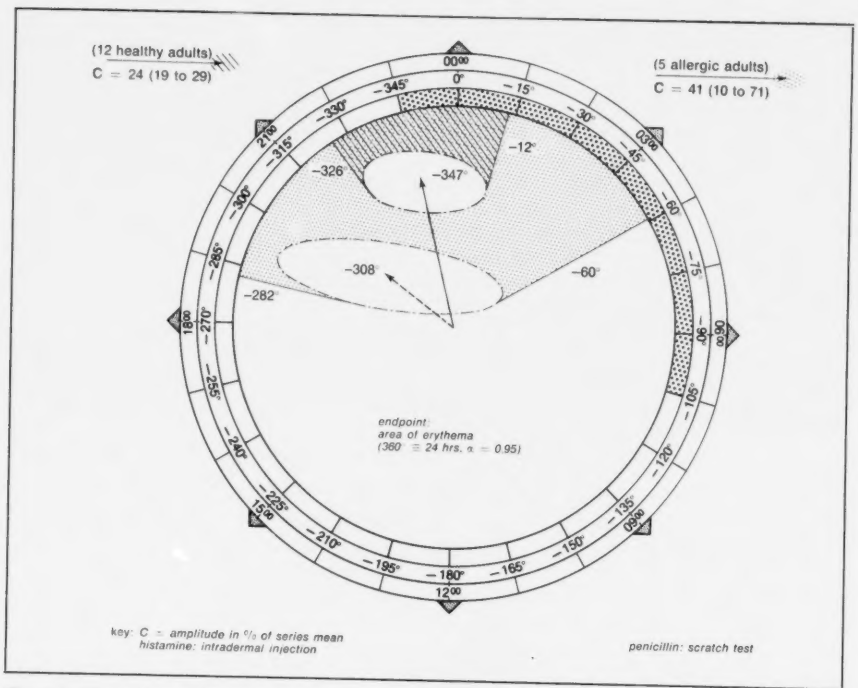


Fig. 25 - Circadian rhythm in skin reactions to histamine ($P = 0.001$) and to penicillin ($P < 0.04$).

Autorhythmometry or rhythmometry for population regulation is likely to become acceptable in proportion to the rigor of its results and the extent to which data collection and analysis can be automated.

Autorhythmometry and chronotherapy

Since the human response to external stimuli (fig. 25) [79] is not the same along a rhythm's time scale [77], clinical investigators and scientists should test means for optimizing drug effects by timing their administration. Methods of analyses available for the study of bioperiodicity have been used as experimental approaches in chronopharmacology: it has been shown that the administration of a potentially harmful yet otherwise useful agent such as a drug has drastically different effects along the 24-h scale, fig. 26. Information about the circadian variations of the interrelationship between toxic effect and therapeutic effect also has accumulated for the case of chemotherapy with ara-C, figs 27a, b, c [38]. Conventional drugs such as ara-C or radiation may be administered at a time when the therapeutic effect is maximal and the toxic effect minimal.

In some cases the usual time relations of rhythm(s) in the curative and toxic effects are such that, without further manipulation, an optimal time is found when the desired effect is maximal and the undesired one minimal.

In other cases it may well be that one could selectively change the usual time relations by scheduling meals [69], or other aspects of life style and/or drugs [30] to bring about an optimized therapeutic ratio at a specifiable and, if possible, convenient

time. Verification of such timing may well be achieved by self-measurement and/or automatic rhythmometry on variables that serve as so-called 'marker' functions. Excretory products of a tumor and its temperature may be marker functions of certain cancers [94], to cite but one example currently under investigation. These aspects of a chronotherapy involve currently conventional treatments that are known to lead to toxicity, as in the corticosteroidal treatment of many conditions and in cancer chemotherapy with ara-C [51] or cyclophosphamide [49].

There remains the possibility that rhythm alteration, beyond being merely associated with disease, may actually determine illness, or at least shorten life span. In such cases (and 'essential' *mesor* hypertension may be a case in point) it may be found that a specially designed (rhythm-alteration correcting) chronotherapy is the most effective treatment. The extent to which such chronopathology can be identified will depend upon the large-scale availability of tools for self-measurement and automatic registration as well as for data transfer and appropriate analyses on rhythmic functions, whether this be done for assessing health, risk or disease.

Status quo

The study of biologic rhythms has progressed through several stages. For all too long a time, rhythms have been regarded as *trivial*. So-called 'diurnal' variations unqualified as to conditions of observation (fig. 28) can be quite confusing. Modern rhythmometry has little to do with an uncritical association of certain values with certain dates or times of day.

In a second stage of chronobiology, the detection of circadian desynchronization led in the early fifties to the analogy of endogenous rhythms with a free-running oscillator [22, 72]. We and many others interpreted the finding of periods that were close to 24 hrs but nonetheless statistically different from precisely 24 hrs as indicative of internal rhythms. Behavior-day charts continue to be published in one form or

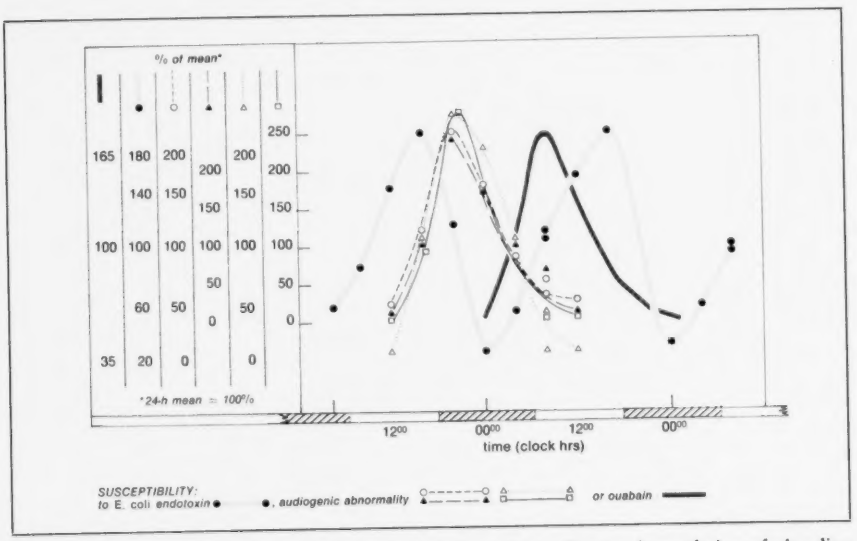


Fig. 26 - Changing resistance to various stimuli during 24 hrs. Temporal correlation of circadian susceptibility rhythms in mice. Standardization in $LD_{12:12}$ for 1 week prior to sampling.

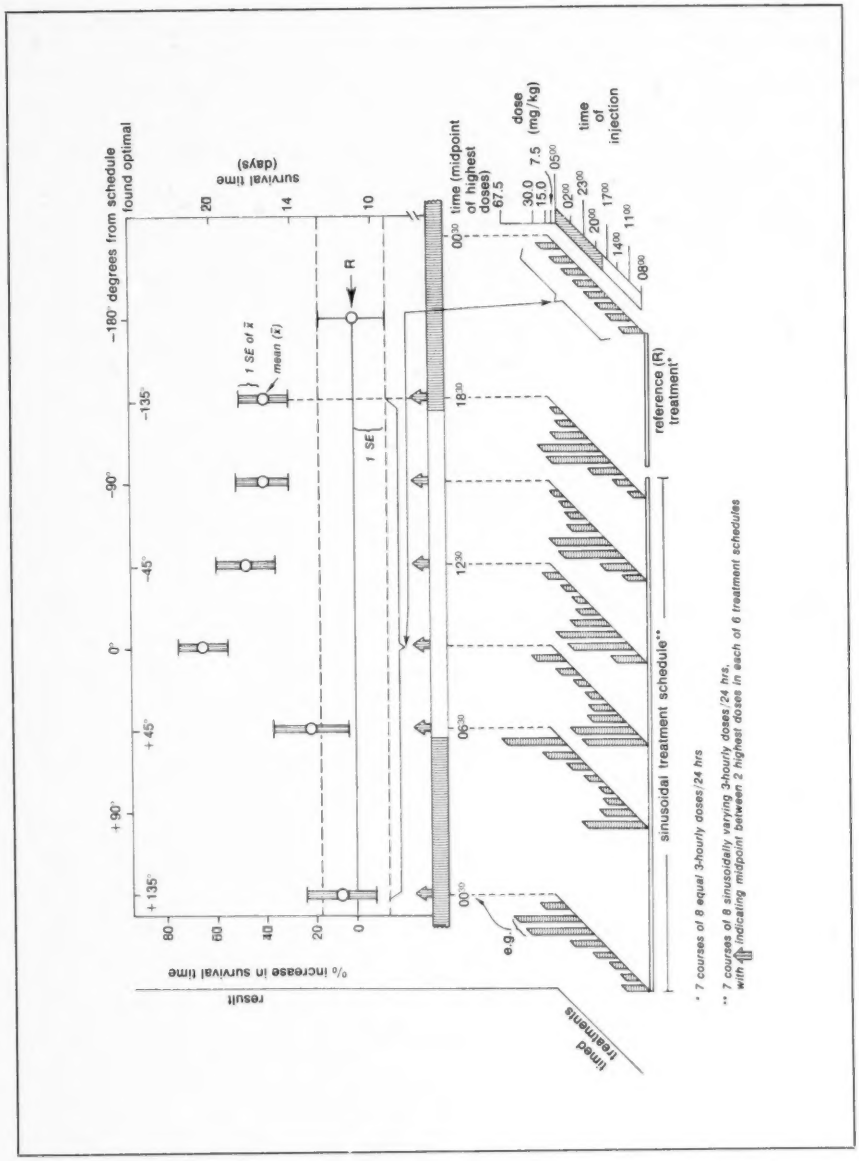


Fig. 27a - Murine tolerance to ara-C on different schedules. Study IV (L1210, inoculation 1/26/1972).

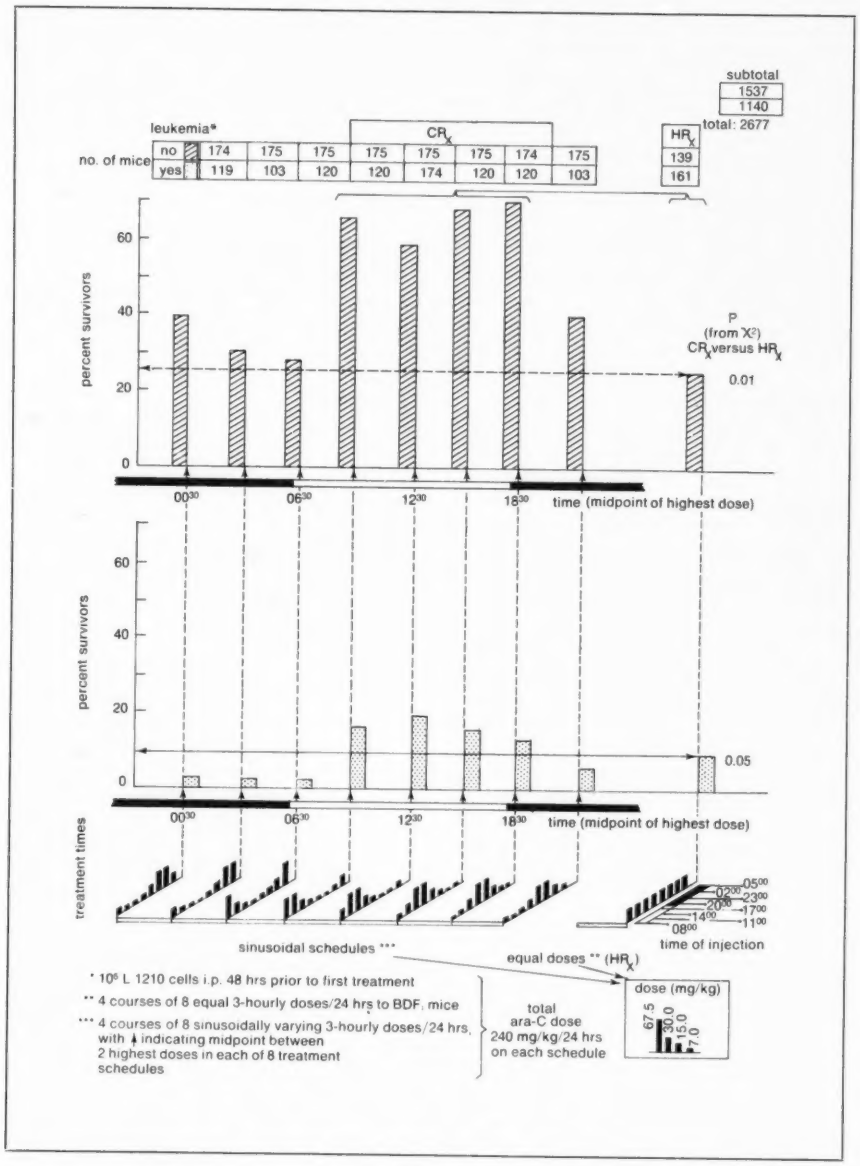


Fig. 27b - Survival of leukemic and non-leukemic mice treated with ara-C on different schedules: chronotherapy (CR_x) *versus* homeostatic therapy (HR_x).

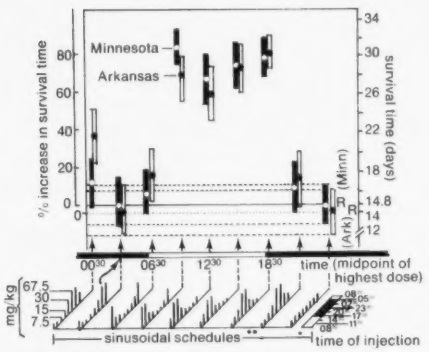
another. More often than not, the total body activity rhythm of a rodent was measured and it was noted that activity onsets started earlier each day or later each day. While initially such findings were indeed a very obvious indication, say after blinding, of non-24-h rhythms demonstrated both in chronograms and in periodograms, the continued publication of activity charts was by no means a substitute for an analysis of physiologic mechanisms. By the same token, very extensive modeling based upon a black box approach, whereby the cellular, endocrine and neural cycles were usually ignored, did not lead to the solution of problems, and perhaps did not even help to approach such solutions.

Accordingly, the broader medical community has come to regard such findings of 'free-running' rhythms as basic yet *esoteric*, since the association of any desynchronized rhythm with a free-running 'clock' remains of questionable heuristic value. Not only the quantification of contributions by variations with periods different from precisely 24 hrs (fig. 29, right-hand section), rather than their description in a behavior-day chart (fig. 29), can be advocated in this connection, but also the exploitation of rhythms for applied as well as basic knowledge.

Conceivably, in a third stage, rhythms will be recognized as *important* and *useful*, rather than as esoteric or trivial entities. We need only recognize in clinical practice the need to avoid undue toxicity from chemotherapy that is already rigorously documented for the experimental animal. The extent to which investigative rhythmometry will be useful and sufficient to establish group schedules for treatment and/or the need for continued use of AR to assess individualized physiologic markers for chronotherapy, e.g. in treatment of human cancer, await clinical investigation. By the same token, autorhythmometry with a peak flowmeter can be advocated at least for assessing the effect of treatment in asthma [80] and, perhaps, for timing treatment with steroids in order to achieve a maximal desired effect [78].

The exactitude of autorhythmometry has been justly questioned. Nevertheless it is heartening to realize that good agreement has been found between medical school staff and groups of 20 to 50 high school students who had a single introduction to blood pressure measurement. The agreement between staff and student was excellent when both listened to the same sounds and separately wrote down the results. Another criticism voiced relates to the accumulation of large batches of data that may be difficult to interpret. Yet it is taken for granted that extensive data must be accumulated for billing patients in hospitals. A system which seems to be essential to avoid or at least delay the need for such bills by monitoring health should have equal priority.

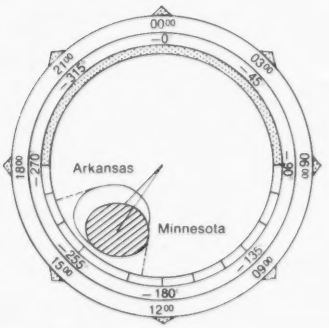
Fig. 27c - *Top*: survival time of $CD2F_1$ mice on different drug administration schedules (top) and timing of doses of ara-C (bottom) in sinusoidal and reference (R) schedules consisting of 4 courses of 240 mg/kg/24 hrs each. When the same total dose of ara-C is given, certain sinusoidal drug administration schedules are definitely better tolerated by mice than are other sinusoids or a currently conventional reference treatment of 8 equal doses over a 24-h span. Also note unequivocal reproducibility of chronotoxicity to ara-C in experiments done on the same days in different laboratories in different geographic locations. *Bottom*: *cosinor* summary of data of the experiments on the top. Polar coordinates are used to display in inferential statistical terms an extent of change of a biological rhythm (amplitude) and timing of change (acrophase) relations by the length and the angle of a directed line, respectively, shown with a bivariate statistical confidence region computed at a fixed (in this case 24-h) trial period, first to detect a rhythm by a confidence region not overlapping the pole and second to estimate confidence intervals for the rhythm parameters. Thus, a measure of dispersion for the estimate of timing is given by *radii* drawn tangent to the confidence regions. The assumption underlying such a test is that the series summarized are comparable in terms of conditions of observations and/or individuals and that they are characterized by a rhythm with a similar frequency and timing. This assumption is extremely well validated (for the 2 sets of data analyzed) insofar as timing is concerned. The confidence regions of timing are similar, and the point estimates of timing vary by much less than 1 h (since 360° are equated to 24 hrs, 10° are equated to 40 min). (SCHEVING L. E. et al. [82]). ▶



* 4 courses of 8 equal 3-hourly doses/24 hrs
** 4 courses of 8 sinusoidally varying 3-hourly doses/24 hrs, with ▲ indicating midpoint between 2 highest doses in each of 8 treatment schedules

ara-C (240 mg/kg/24 hrs)

mean: SE



	mesor (M)* M ± SE	amplitude (A)* A ± SE	acrophase (∅)** ∅	95% CI	% rhythm	P - value F - test
Minnesota	554 ± 22	206 ± 31	−211°	−188°, −233°	22	01
Arkansas	560 ± 24	167 ± 34	−221°	−191°, −251°	13	01

N = 180 mice (20/time point) in each location

* in hrs
** from local midnight

As autorhythmometry progressed from a sample of 20 to a sample of 100 students in some Minnesota high schools [37] and to a sample of 35 in Connecticut, USA [61], additional steps for validating its practicability for a given setting have been achieved. However, practicability on a much larger scale and for different population groups, such as urban and rural poor, employee groups, and hospital and clinic patients remain to be separately tested. Follow-up steps for cohorts of persons who already have practiced autorhythmometry will have to be specified and implemented.

Moreover, with an appropriate system for analysis, epidemiologic information on the natural history of the disease should become available for creating models of the human conditions in the form of *mesor*-hypertensive and other animals. In *mesor*-hypertensive and more so in other models, such as in the case of an infradian amplitude elevation, the question as to the extent to which an alteration in a circadian or ultradian rhythm's amplitude and/or acrophase precedes the onset of infradian *mesor*-hypertension remains to be scrutinized.

Against the background of such information, yet to be collected, we shall have to ask whether autorhythmometry will be effective in overall health care. Logic dictates an affirmative answer, but in view of our limited health resources, we then confront an added question, as we do in the case of transplants. More specifically, a procedure has to be more useful than others; it should benefit more people than competing endeavors and, if possible, it should not strain the currently precarious state of the national economy. From all of these viewpoints, AR seems to offer 'more for less'. Autorhythmometry compares particularly favorably with available alternatives, i.e. intervention only after a complaint arises or after the customary single sample check-up reveals an alteration or risk, or a disease is established: one needs only com-

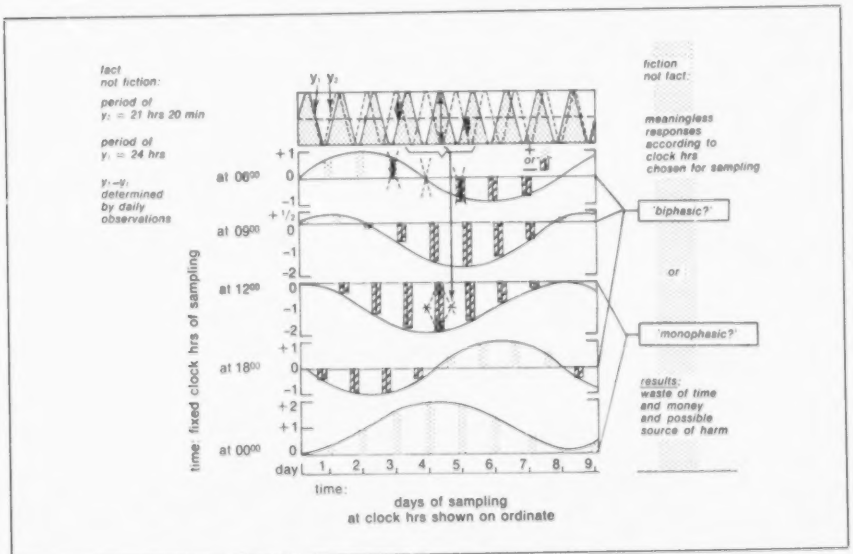


Fig. 28 - Pitfalls of mistaking control of time of day for control of rhythms. Responses consisting of a decrease *or* increase (often inappropriately described as *monophasic*) or responses with both a decrease *and* an increase (often inappropriately described as *biphasic*) can be obtained. Such artifacts must be avoided. However, rhythmometry can be advocated because it opens a new dimension for preventive medicine, diagnosis and treatment (rather than solely because it will help avoid confusion).

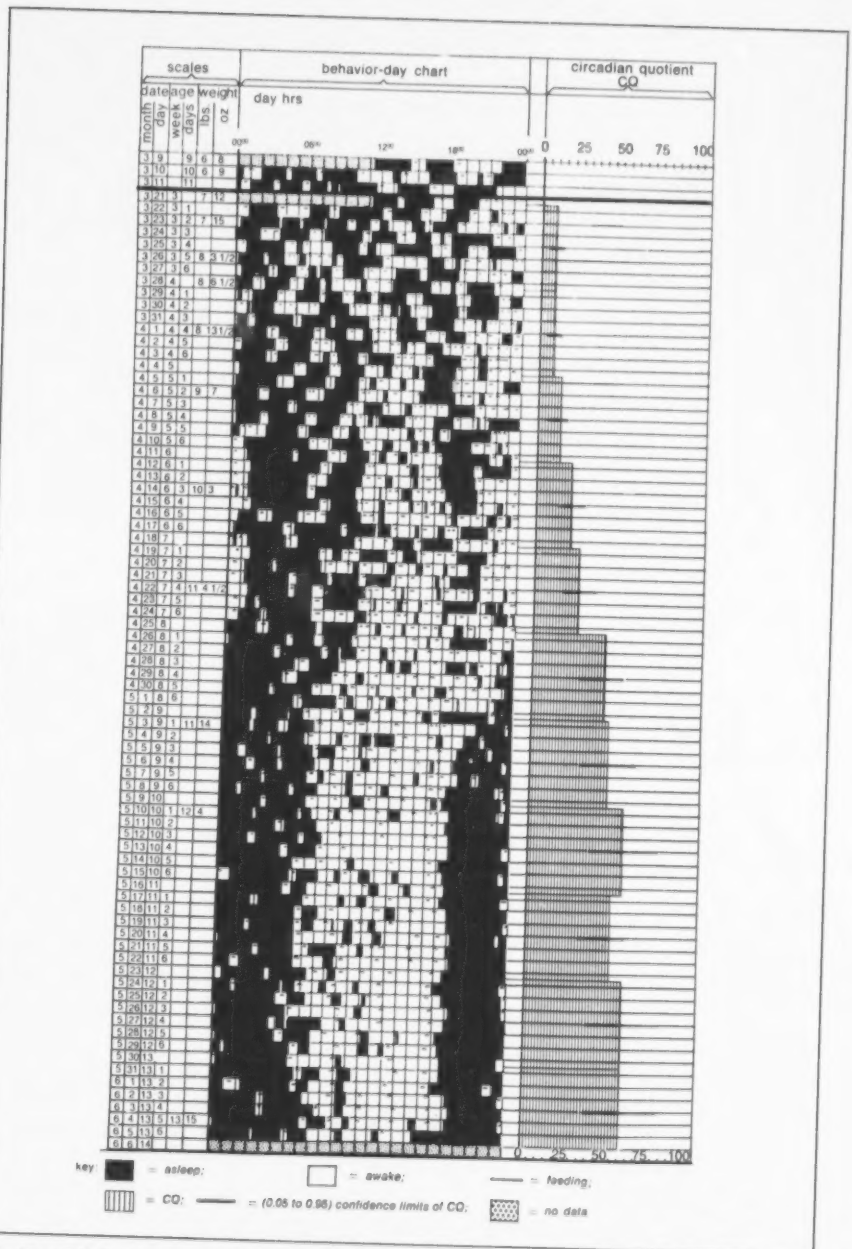


Fig. 29 - F.A.R., born on 2/28/1951 (0155); birth weight: 6 lbs. 8$^1/_2$ oz. Behavior-day chart (*on the left*) quantified by power spectral analysis, providing the circadian quotient as a derived measure for assessing the prominence of the circadian rhythm as it develops in a male infant on self-demand.

pare its cost with that of maintaining patients after a stroke or coronary diseases. In contrast to conventional screening (after which the information obtained is discarded), AR aims at obtaining and storing for future reference information from the unworried healthy individual as well as from the one who is healthy but worried. AR serves to collect data about such persons for use as individualized reference standards in health. Thus, AR promises to provide more pertinent and almost certainly more sensitive criteria for evaluating health than do other methods used in screening. It undoubtedly provides more effective screening in recognizing, *inter alia*, odd-hour-hypertension or silent stages of an intermittent ('labile') blood pressure elevation that could not be detected at a single check-up during 'office hours'.

SUMMARY

Starting from the definition of health as given by the WHO, the authors emphasize that rhythms of biological variables should be taken into account if one is to arrive at a positive and individual definition of the concept of health. The evaluation of a single datum spot check of physiological functions does not permit to quantify health in dynamic terms whereas this may become possible when the rhythmic quality of these variables, according to a spectrum of characteristic frequencies, has been assessed. Rhythmometry provides for appropriate reference standards as regards both the population in general and the physical conditions of the same subject during certain spans in the (prospective) life history (spans selected according to consideration of risk and hence cost and benefit at a given age). The authors therefore stress the importance of rhythmometry, thanks to which the time course and other peculiarities of any biological phenomenon can be evaluated by obtaining serial measurements, objectively quantifying their characteristic features, and working out special models with the aid of computers. Of special interest, particularly for the evaluation of reference standards, is autorhythmometry (AR), to be used at least for certain variables. This is the method by which each subject studies himself, performing a certain number of measurements in the course of the day (or month or year) of his body temperature, blood pressure, cardiac rhythm, grip strength, etc. In AR, the subject takes an active part in the study of his condition of health and it has been shown that in the majority of cases these measurements are done with the utmost accuracy and precision, providing that the purpose of the operations to be performed has been adequately explained. The wide spread use of AR, during more or less extended periods in a lifetime, would permit the assessment of the *normal situation* for each individual and thus offer the possibility of diagnosing any disorder at its very beginning; it would thus be of great prophylactic value. The authors refer to the example of arterial blood pressure; widely different values for the normal levels can be found in the texts of different authors. They also show that a certain pressure may be normal for a given subject at a certain time of day and be a sign of illness for another subject, or even for the same subject at a different time. The authors suggest the introduction of AR in the *curriculum* of secondary schools and into the routine for outpatients and inpatients. This practice would increase the availability of information on public health at a lower cost. AR may be very usefully applied to chronotherapy: it is a well-known fact that there are rhythms of sensitivity and therefore rhythms of toxic as well as therapeutic effects for various drugs. These can be dove-tailed in such a way as to work out, for individual cases, a time for administration at which the therapeutic effect is at its maximum and the toxic one at its minimum. If this is not the case, it may be useful to modify some features of these rhythms, e.g. by shifting meal times, so as to obtain an optimal therapeutic index.

REFERENCES

1) Apfelbaum M., Bishop J. S., Cressey D., Davies G., Doe R., Goetz F. C., Halberg F., Haus E., Kramm K., MacDonald J., Nelson W., Reinberg A., Scheving L. E., Simpson H.: Human or Murine Endocrine and Metabolic Rhythms after Changes in Meal Timing with or without a Fixed Activity Schedule - In: Proceedings of the Fifty-Sixth Meeting of the Endocrine Society; Abstract No. 308, 1974.
2) Aschoff J.: Exogenous and Endogenous Components in Circadian Rhythms - In: Cold Spring Harbor Symposia on Quantitative Biology. Long Island Biol. Ass. N.Y., 1960; p. 11.
3) Aschoff J.: Physiologie biologischer Rhythmen - Ärztl. Praxis *18*, 1, 1966.
4) Aschoff J.: Desynchronization and Resynchronization of Human Circadian Rhythms - Aerospace Med. *40*, 844, 1969.
5) Aschoff J., Wever R.: Resynchronisation der Tagesperiodik von Vögeln nach Phasensprung des Zeitgebers - Z. vergl. Physiol. *46*, 321, 1963.

6) BARNUM C. P., JARDETZKY C. D., HALBERG F.: Time Relations among Metabolic and Morphologic 24-Hour Changes in Mouse Liver - Amer. J. Physiol. *195*, 301, 1958.
7) BARTTER F. C.: Periodicity and Medicine - In: SCHEVING L. E., HALBERG F., PAULY J. E. (Eds): Chronobiology. Igaku Shoin Ltd., Tokyo, 1974; p. 6.
8) BERNARD C.: De la diversité des animaux soumis à l'expérimentation. De la variabilité des conditions organiques dans lesquelles ils s'offrent à l'expérimentateur - J. Anat. (Paris) *2*, 497, 1865.
9) BÜNNING E.: The Physiological Clock - Springer-Verlag, Berlin, 1964; p. 145.
10) CARANDENTE F., HALBERG E., DUBEY D. P., BEARD K., KLEIN P., HALBERG F.: Chronobiology and Sex as Codeterminants of Gastric Ulcerogenesis in 'Loaded' Rats of Two Inbred Strains - In: Proceedings of XII International Conference of International Society for Chronobiology. August 10-13, 1975 - Chronobiologia *3* (Suppl.), 1976. (In Press).
11) CHIBA Y., CUTKOMP L. K., HALBERG F.: Circaseptan (7-Day) Oviposition Rhythm and Growth of Spring Tail, *Folsomia candida (Collembola: Isotomidae)* - Int. J. Cycle Res. *4*, 59, 1973.
12) CHIBA Y., CUTKOMP L. K., HALBERG F.: Circadian Oxygen Consumption Rhythms of the Flour Beetle, *Tribolium confusum*, with Special Reference to Phase-Shifting - In: SCHEVING L. E., HALBERG F., PAULY J. E. (Eds): Chronobiology. Igaku Shoin Ltd., Tokyo, 1974; p. 602.
13) CHOSSAT C.: Recherches expérimentales sur l'inanition - Mém. Acad. roy. Sci. Inst. France *8*, 438, 1843.
14) COLOMBO C.: Recherches sur la pression du sang chez l'homme - G. Accad. Med. Torino *62*, 23, 1899.
15) CROWLEY T. J., KRIPKE D., HALBERG F., PEGRAM G. V., SCHILDKRAUT J. J.: Circadian Time Relations of Simian Brain Temperature, Sleep, EEG, EMG, Body and Eye Movement - Prim. Med. (Basel) *13*, 149, 1972.
16) DARWIN C.: The Descent of Man. London, 1871; vol. 1, p. 212.
17) DESCOVICH G. C., MONTALBETTI N., KÜHL J. F. W., RIMONDI S., HALBERG F.: Circadian as well as Circannual in-Phase Synchronization of Urinary Epinephrine (E) and Norepinephrine (NE) and Acrophase-Difference of E or NE with NER = NE/(NE + E) in Mature and Presumably Healthy Human Beings - Int. J. Chronobiol. *1*, 324, 1973.
18) DÖRING G. L.: Detection of Ovulation by the Basal Body Temperature Method - In: URICCHIO W. A., WILLIAMS M. K. (Eds): Proceedings of a Research Conference on Natural Family Planning. The Human Life Foundation, Washington, D.C., 1974; p. 171.
19) FERIN M., HALBERG F., RICHART R. M., VANDE WIELE R. L. (Eds): Biorhythms and Human Reproduction. John Wiley and Sons, New York, 1974; p. 665.
20) GAGLIARDINO J. J., HERNANDEZ R. E.: Circadian Variations of the Serum Glucose and Immunoreactive Insulin Levels - Endocrinology *88*, 1529, 1971.
21) GAGLIARDINO J. J., HERNANDEZ R. E.: Relationship between Differential Responsiveness of Pancreatic Beta Cells and the Circadian Variation of Serum Immunoreactive Insulin Levels - Endocrinology *91*, 822, 1972.
22) HALBERG F.: Temporal Coordination of Physiologic Function - In: Cold Spring Harbor Symposia on Quantitative Biology. Long Island Biol. Ass. N.Y., 1960; p. 289.
23) HALBERG F.: Claude Bernard, Referring to an 'Extreme Variability of the Internal *Milieu*' - In: Claude Bernard and Experimental Medicine. Schenkman Publ. Co., Cambridge, Massachusetts, 1967; p. 193.
24) HALBERG F.: Chronobiology - Ann. Rev. Physiol. *31*, 675, 1969.
25) HALBERG F.: Physiologic Considerations Underlying Rhythmometry, with Special Reference to Emotional Illness - In: Symposium on Biological Cycles and Psychiatry, in Symposium Bel-Air III. Genève, Masson et Cie, 1968; p. 73.
26) HALBERG F.: Circadian System of Nonhuman Primates: Summary of a Symposium in 1968 and of Earlier Work - In: ROHLES F. H. (Ed.): Circadian Rhythms in Nonhuman Primates. Karger, Basel/New York, 1969; p. 106.
27) HALBERG F.: Chronobiology and the Delivery of Health Care - In: O'LEARY H. (Ed.): A System Approach to the Application of Chronobiology in Family Practice. Health Care Research Program, Department of Family Practice and Community Health, University of Minnesota, 1970; p. 31.
28) HALBERG F.: Body Temperature, Circadian Rhythms and the Eye - In: BENOIT J., ASSENMACHER I. (Eds): La photorégulation de la réproduction chez les oiseaux et les mammifères. Centre National de la Recherche Scientifique, Paris, No. 172, 1970; p. 497.
29) HALBERG F.: Laboratory Techniques and Rhythmometry - In: MILLS J. N. (Ed.): Biological Aspects of Circadian Rhythms. Plenum Press, London and New York, 1973; p. 1.
30) HALBERG F.: Protection by Timing Treatment According to Bodily Rhythms - An Analogy to Protection by Scrubbing before Surgery. *Tempus non solum dosis venenum facit* - Chronobiologia *1* (Suppl.), 27, 1974.

55) JARRETT R. J.: Diurnal Variation in Glucose Tolerance; Associated Changes in Plasma Insulin, Growth Hormone and Non-Esterified Fatty Acids and Insulin Sensitivity - Chronobiologia *1* (Suppl.), 229, 1974.

56) JONES W. H. S.: Hippocrates with an English Translation - In: PAGE T. E., CAPPS E., PAST L. A., ROUSE W. H. D., WARMINGTON E. H. (Eds): Hippocrates. Harvard University Press, Cambridge, Massachusetts, 1948; p. 360.

57) KIESOW I. V.: Un nuovo metodo per misurare la pressione - Arch. ital. Biol. *28*, 52, 1895.

58) KOUKKARI W. L., HALBERG F.: Quantifying Rhythmic Movements of *Albizzia julibrissin* Pinnules - Plant Physiol. *51*, 1084, 1973.

59) KUZEL M. K.: Circadian and Ultradian Rhythms Identified in Temperature, Blood Pressure, Heart Rate, Dysrhythmias and Susceptibility Cycles Following Acute Myocardial Infarction - M.D. Thesis, DePaul University, 1973.

60) LEVINE H., HALBERG F., SOTHERN R. B., BARTTER F. C., MEYER W. J., DELEA C.: Circadian Phase-Shifting with and without Geographic Displacement - In: FERIN M., HALBERG F., RICHART R. M., VANDE WIELE R. L. (Eds): Biorhythms and Human Reproduction. John Wiley and Sons, New York, 1974; p. 557.

61) LEVINE H., JOHNSON L. R., LASALLE D. P., HALBERG F.: Autorhythmometry by Secondary School Students in Connecticut and Minnesota - In: Proceedings 1st World Congress of Environmental Medicine and Biology, Paris, 1975. (Excerpta med. - In Press).

62) LOGAN R. F. L.: Assessment of Sickness and Health in the Community: Needs and Methods. Parts I and II - Med. Care *2*, 173; 218, 1964.

63) LUCE G. G.: Biological Rhythms in Psychiatry and Medicine - National Institute of Mental Health, Public Health Service Publication No. 2088, US Government Printing Office (Washington, D.C.), 1970; p. 173.

64) LUNEDEI A., CAGNONI M., FANTINI F., TARQUINI B., MORACE G., MAIELLO M., PANERAI A., SCARPELLI P. T., TOCCAFONDI R.: Sindromi diencefaliche (problemi in discussione) - Edizioni L. Pozzi, Roma, 1967; p. 413.

65) MENZEL W.: Menschliche Tag-Nacht-Rhythmik und Schichtarbeit - Benno Schwabe und Co. Verlag, Basel/Stuttgart, 1962; p. 189.

66) MORIYAMA I. M.: Problems in the Measurement of Health Status - In: SHELDON E. B., MOORE W. E. (Eds): Indicators of Social Change. Russell Sage Foundation, New York, 1968; p. 573.

67) NELSON W., HALBERG F., HWANG D. S.: An Evaluation of Time-Dependent Changes in Susceptibility of Mice to Pentobarbital Injection - Neuropharmacology *12*, 509, 1973.

68) NELSON W., HALBERG F., SCHEVING L. E.: Meal Timing as an Adjuvant of Experimental Chronotherapy: Circadian Rhythm in Tolerance of Adriamycin by Mice on a 24-Hour Cyclic Lighting Regimen — the Usually Dominant Synchronizer Routine — Is Shifted by Restricting Feeding to Early Light Span - In: XI International Cancer Congress, 1974, Florence, Italy. Chronobiologia *1*, 315, 1974.

69) NELSON W., SCHEVING L. E., HALBERG F.: Circadian Rhythms in Mice Allowed Single Daily 'Meal' at Different Stages of LD (12:12) Lighting Regimen - J. Nutr. *105*, 171, 1975.

70) PARSONS T.: Definitions of Health and Illness in the Light of American Values and Social Structure - In: GARTLY JACO E. (Ed.): Patients, Physicians and Illness. The Free Press, Glencoe, Illinois, 1958; p. 165.

71) PICKERING G.: Hypertension. Causes, Consequences and Management - J. and A. Churchill, London, 1970; p. 28.

72) PITTENDRIGH C. S.: Circadian Rhythms and the Circadian Organization of Living Systems - In: Cold Spring Harbor Symposia on Quantitative Biology. Long Island Biol. Ass. N.Y., 1960; p. 159.

73) REINBERG A.: Ritmi biologici naturali e condizionati nell'uomo - Enciclopedia della Scienza e della Tecnica Mondadori *11*, 148, 1968.

74) REINBERG A., CLENCH J., AYMARD N., GALLIOT M., BOURDON R., GERVAIS P., ABULKER CH., DUPONT J.: Physiologie - rythmes circadiens des paramètres de l'ethanolémie provoquée chez six hommes adultes, jeunes et sains - C.R. Acad. Sci. (Paris) *278*, 1503, 1974.

75) REINBERG A., GERVAIS P., ROYNETTE N., ABULKER CH., GAULTIER M., HALBERG F.: Rythmes circadiens et circannuels de mortalité des adultes en France et à l'hôpital Fernand Widal (Paris) - Nouv. Presse méd. *2*, 289, 1973.

76) REINBERG A., GHATA J.: Biological Rhythms - Walker, New York, 1964; p. 138.

77) REINBERG A., HALBERG F.: Circadian Chronopharmacology - Ann. Rev. Pharmacology *2*, 455, 1971.

78) REINBERG A., HALBERG F., FALLIERS C.: Circadian Timing of Methylprednisolone Effects in Asthmatic Boys - Chronobiologia *1*, 333, 1974.

79) REINBERG A., ZAGULA-MALLY Z., GHATA J., HALBERG F.: Circadian Reactivity Rhythm of Human Skin to House Dust, Penicillin and Histamine - J. Allergy *44*, 292, 1969.

80) Reindl K., Falliers C., Halberg F., Chai H., Hillman D., Nelson W.: Circadian Acrophases in Peak Expiratory Flow Rate and Urinary Electrolyte Excretion of Asthmatic Children; Phase-Shifting of Rhythms by Prednisone Given in Different Circadian System Phases - Rass. Neurol. veg. *23*, 5, 1969.

81) Rutenfranz J., Singer R.: Untersuchungen zur Frage einer Abhängigkeit der Alkoholwirkung von der Tageszeit - Int. Z. angew. Physiol. *24*, 1, 1967.

82) Scheving L. E., Haus E., Kühl J. F. W., Pauly J. E., Halberg F., Cardoso S. S.: Close Reproduction by Different Laboratories of Characteristics of Circadian Rhythm of 1-β-D-Arabinofuranosylcytosine Tolerance by Mice - Cancer Res. *36*, 1133, 1976.

83) Scheving L. E., Pauly J. E., Burns E. R., Halberg E., Tsai S., Betterton H. O.: Lighting Regimen Dominates Interacting Meal Schedules and Synchronizes Mitotic Rhythm in Mouse Corneal Epithelium - Anat. Rec. *180*, 47, 1974.

84) Schmitt O.: The Personalized Portable Whole-Life Medical System - Clin. Engin. News *3*, 1, 1975.

85) Shiotsuka R., Halberg F., Haus E., Lee J. K., McHugh R., Simpson H., Levine H., Ratte J., Najarian J.: Results Bearing on the Chronotherapy of Hypertension: Saluresis and Diuresis without Kaluresis Can Be Produced by Properly Timing Chlorothiazide Administration According to Circadian Rhythms - Int. J. Chronobiol. *1*, 358, 1973.

86) Siegel M. P.: Scientific Management of Population Growth - Vital Speeches of the Day *40*, 603, 1974.

87) Simpson S., Galbraith J. J.: Observations on the Normal Temperature of the Monkey and its Diurnal Variation, and on the Effect of Changes in the Daily Routine on this Variation - Trans. roy. Soc. (Edinburgh) *45*, 65, 1905.

88) Smolensky M., Halberg F., Sargent II F.: Chronobiology of the Life Sequence - In: Ito S., Ogata K., Yoshimura H. (Eds): Advances in Climatic Physiology. Igaku Shoin Ltd., Tokyo, 1972; p. 281.

89) Takebe K., Setaishi C., Hirama M., Yamamoto M., Horiuchi Y.: Effects of a Bacterial Pyrogen on the Pituitary-Adrenal Axis at Various Times in the 24 Hours - J. clin. Endocr. *26*, 437, 1966.

90) Tarquini B., Della Corte M., Orzalesi R.: Circadian Studies on Plasma Cortisol in Subjects with Peptic Ulcer - J. Endocr. *38*, 475, 1967.

91) Wilson R. H. L., Newman E. J., Newman H. W.: Diurnal Variation in Rate of Alcohol Metabolism - J. Appl. Physiol. *8*, 556, 1956.

92) World Health Organization Official Records: 'Final Acts', from the Constitution Signed July 22, 1946; p. 100.

93) Yunis E. J., Fernandes G., Nelson W., Halberg F.: Circadian Temperature Rhythms and Aging in Rodents - In: Scheving L. E., Halberg F., Pauly J. E. (Eds): Chronobiology. Igaku Shoin Ltd., Tokyo, 1974; p. 358.

94) Zinneman H. H., Halberg F., Haus E., Kaplan M.: Circadian Rhythms in Urinary Light Chains, Serum Iron and Other Variables of Multiple Myeloma Patients - Int. J. Chronobiol. *2*, 3, 1974.

Requests for reprints should be addressed to:

Franz Halberg

380 Lyon Laboratories
Chronobiology Laboratories
Department of Laboratory Medicine and Pathology
University of Minnesota
Minneapolis, Minnesota 55455, USA